AN INVESTIGATION OF NITROGEN CYCLING PROCESSES IN A COASTAL FYNBOS ECOSYSTEM IN THE SOUTH WESTERN CAPE PROVINCE, SOUTH AFRICA.

By

WILLIAM DAVID STOCK

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ABSTRACT

The Cape Floral Kingdom, known locally as "fynbos", is of great scientific and aesthetic interest as well as being economically important as a water source zone, as a source of flowers for the cut flower industry and as a recreation area. Sound ecological knowledge is required in order to manage and conserve fynbos because the extent of this unique, species-rich, endemic flora has been drastically reduced. Nutrients, in particular nitrogen and phosphorus, have been identified as being of crucial importance in the structure and functioning of fynbos ecosystems because of the nutrient poor substrates upon which this vegetation type exists.

This study has concentrated on the following aspects of the nitrogen cycle which are considered to be important in describing the functioning of a coastal fynbos ecosystem:

a) The nitrogen status of soils supporting coastal fynbos.
b) The forms of nitrogen and the seasonal changes in nitrogen concentrations in these soils.
c) The influence of fire disturbance on nitrogen form and concentration in these soils.
d) An investigation of the nitrogen mineralization process in these soils in relation to control by physical factors, successional age of the stand and the impact of fire on this process.
e) The uptake and utilization of different forms of nitrogen by characteristic species of fynbos vegetation.

f) The importance of internal recycling of nitrogen as an adaptation to the low nutrient soils of the area.

g) The role of atmospheric inputs of nitrogen to the ecosystem, in particular the importance of this source in replacing nitrogen lost during recurrent fires.

The levels of soil nitrogen (both total and inorganic nitrogen) investigated at the Pella intensive study site were found to be low. The small significant seasonal variations found in inorganic nitrogen concentrations were indicative of an undisturbed natural ecosystem which has a very efficient 'tight' nitrogen cycle. The mineralization of organic nitrogen was found to be a process greatly influenced by the environmental controls typical of the mediterranean climate. Successional age of the community was not found to influence the nitrification step of the mineralization process. This has previously been suggested as a nitrogen conservation mechanism by Rice and Pancholy (1972) as it is thought to prevent nitrogen losses from leaching of nitrate. The low nitrogen concentrations of the sandy soils and the environmental factors peculiar to the mediterranean climate result in the mineralization process probably being the rate-limiting process controlling nitrogen turnover and potential productivity of undisturbed coastal fynbos.

Plant species of coastal fynbos vegetation investigated revealed a number of adaptations characteristic of species from low nutrient environments. Plants such as members of the Proteaceae are known to have mechanisms to improve nutrient uptake, such as proteoid roots. They were shown in this study

to have low nitrogen metabolic requirements attuned to the low nitrogen supply of the soils. The low metabolic requirement was demonstrated by exceedingly low levels of nitrate reductase activity and poor assimilation potentials for both $^{15}NO_3$-N and $^{15}NH_4$-N. Other species such as Thamnochortus punctatus (Restionaceae) were found to be efficient in conserving nitrogen as between 35 and 70% of total nitrogen within culms and branches was withdrawn before organ death and reallocated to developing plant parts. This species also showed an asynchronous organ growth pattern where existing nitrogen capital was utilized for several functions during the growing season.

Deposition of nitrogen from the atmosphere to coastal fynbos was low (1,99 kg total N ha^{-1} y^{-1}). The significance of this low input in plant nutrition in the region remains unknown but it may contribute up to 17% of the annual quantity of soil nitrogen made available to plants.

Studies of the effect of fire on soil nitrogen showed fire to be an effective, if destructive, mineralizing agent which causes a release of available nutrients into the system. Direct effects of fire were evident as increased concentrations of total nitrogen (increase of 66 kg ha^{-1}) and ammonium at the soil surface. Nitrate did not increase immediately as a result of the fire but increased quantities were apparent for 9 months after the fire. No evidence of fire destruction of allelochemical nitrification inhibitors was shown. The importance of nitrogen input from precipitation in replenishing nitrogen lost to the

ecosystem by volatilization during fire was small as it would take between 10-80 years to replace ecosystem nitrogen losses by this process. The lenth of time to replace nitrogen from this source is greater than the present fire cycle in fynbos ecosystems which is between 12-15 years.

The findings of the study reported in this thesis were integrated with existing literature to produce an account of nitrogen cycling in this region. These findings were also discussed in relation to future conservation and management of this unique floral kingdom.

ACKNOWLEDGEMENTS

This study could not have been completed without the assistance of others. I therefore would like to express my thanks and appreciation to the following persons and institutions for their help, guidence and encouragement.

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6) To Jaqui Sommerville and Nicky Allsopp your collaboration on different parts of the project is greatly appreciated.

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PREFACE

The investigation reported upon in this thesis was carried out in the Botany Department of the University of Cape Town as part of the Fynbos Biome Project co-ordinated by the Co-operative Scientific Programmes Section, Foundation of Research and Development of the Council for Scientific and Industrial Research, South Africa.

The main objective of the project was to investigate nitrogen cycling processes considered to be important in future management of fynbos vegetation. Studies were conducted in three separate areas of the nitrogen cycle, namely in the soil, the plant and the atmosphere. The thesis is structured into chapters, each dealing with a specific aspect of nitrogen cycling. These chapters are structured as papers for publication, each with its own introduction and discussion. A general introduction to the thesis is given to introduce the background and rationale of the project. A general discussion concludes the thesis by summarizing the major findings of the study.

The following investigations of N cycling processes in coastal fynbos ecosystems are reported on in this thesis:-

1) The nitrogen status of the sandy soils supporting coastal fynbos vegetation at the Pella research site.
2) The forms of nitrogen and seasonal changes in nitrogen concentrations in these soils.
3) The influence of fire disturbance on nitrogen form and concentration in these soils.
4) Nitrogen mineralization processes in these soils (in order to

determine the importance of climatically controlled physical factors, the successional age of the stand and the role of fire in affecting the release of mineral nitrogen by this biological process).

5) The uptake and utilization of different forms of nitrogen by plants characteristic of fynbos vegetation.
6) The importance of internal recycling of nitrogen as an adaptation to the low nutrient soils of the area.
7) The importance of atmospheric inputs of nitrogen to a low nutrient system such as this and to investigate how important this source is in replacing nitrogen lost from recurrent fires.

A comparision of the results obtained in this study with data for nitrogen cycling processes in other mediterranean ecosystems of the world, particularly the Australian mediterranean region which is thought to be most similar in nutritional status to South African fynbos, has also been provided.

Three appendices are attached to this thesis. All three comprize published papers which have been published during the course of the study.

CONTENTS

CHAPTER 1

INTRODUCTION

The sclerophyllous heathlands and shrublands of the mediterranean-climate zone of the South Western Cape Province, South Africa, form part ot the phytogeographical region termed Capensis (Weimarck 1941, Taylor 1978). This area may be equated with the Cape Floral Kingdom as defined by Takhtajan (1969) and Good (1974) and is the smallest of the six world floristic kingdoms (Hall 1978) as it covers only 0,04% of the earth's surface.

The Cape Floral Kingdom is known locally as "fynbos" (Moll & Jarman 1984) and is of great scientific and aesthetic interest as well as being economically important as a water source zone, as a source of flowers for the cut flower industry and as a recreation area. Sound ecological knowledge is urgently required for the management of fynbos because the extent of this unique, species rich and endemic flora has been drastically reduced, from an original area of 67 000 km^2 to a present area of 42 000 km^2 (Jarman 1982, Moll & Bossi 1984) owing to human pressures, the incidence of fire and the ingress of alien plant invaders. Ecological studies in the fynbos biome have until recently concentrated on vegetation classification (Taylor 1978, Kruger 1979), vegetation mapping (Acocks 1953, Moll *et al*. 1984) and studies of community patterns and species diversity (Kruger & Taylor 1978, Campbell & van der Meulen 1980, Campbell 1983, Cowling & Campbell 1983, Sommerville 1983). It was with the advent of the Fynbos Biome Co-operative Project (FRD,CSIR, Pretoria) that eco-physiological investigations of fynbos

vegetation were initiated, in particular nutrient cycling studies.

Nutrients were identified as being of crucial importance in the structure and functioning of fynbos vegetation because of the nutrient-poor substrates upon which this vegetation type exists (Cowling & Campbell 1980, Campbell 1983). The role of nutrients in the structure and function of the five relatively small mediterranean-climate zones of the earth, each with its distinct flora and fauna, was futher highlighted at the Third Intenational Conference on Mediterranean Ecosystems Kruger et al. (1983). At this conference the concept of ecological convergence between all mediterranean ecosystems was examined and the central hypothesis of evolutionary convergence considered, namely that "very similar physical environments, acting on phylogenetically dissimilar organisms in different parts of the world, will produce structrally and functionally similar ecosystems " (Cody & Mooney 1978). Initial work in the Mediterranean Basin, Chile and California provided support for the convergence hypothesis (Mooney & Dunn 1970, di Castri & Mooney 1973, Cody & Mooney 1978), but subsequently it has been shown that the above systems differ in certain aspects from the very similar South African and Australian mediterranean ecosystems. The differences have been ascribed to divergent evolutionary histories and the marked difference in edaphic nutrient status (Specht 1979, Kruger et al. 1983).

It has been suggested that in both South African and Australian mediterranean ecosystems the nutrient elements,

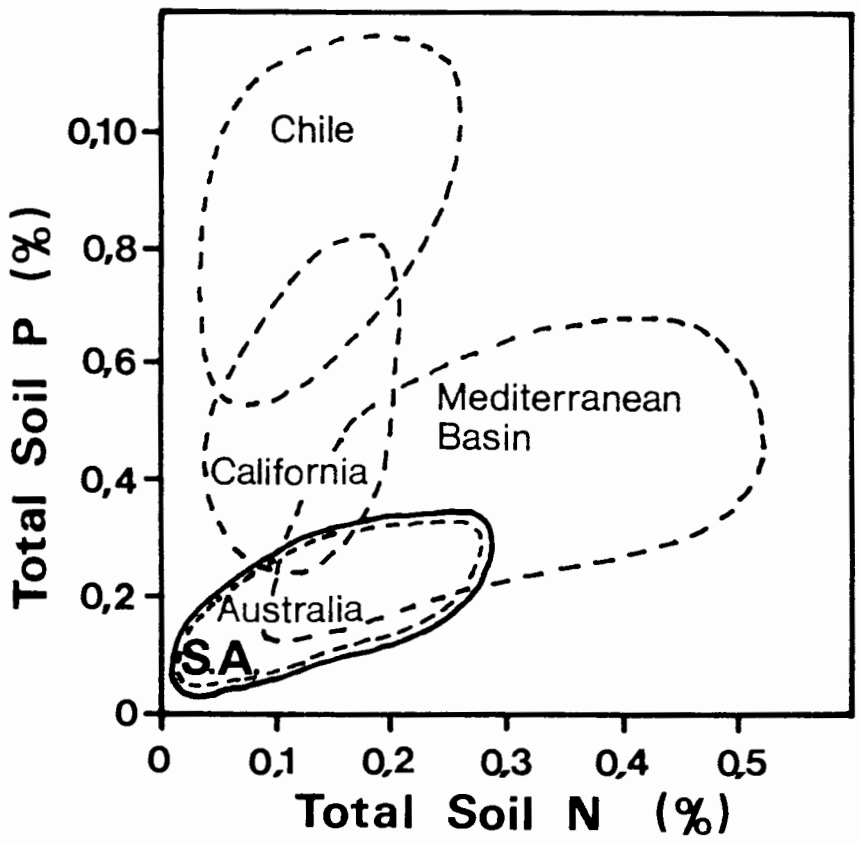


Figure 1.1: Nutrient levels of surface soils in mediterranean regions based on total nitrogen (H_2SO_4 extraction) and total phosphorus (HCl extraction) (after Di Castri 1981, Specht 1981, Groves et al. 1983).

nitrogen and phosphorus, are in particularly low supply (Fig. 1.1) (Wild 1958, Specht 1979, Read & Mitchell 1983). In the Californian mediterranean region it has been shown that the chaparral is moisture and nutrient limited (Hellmers et al. 1955) with low levels of nitrogen cited as one of the major nutrient shortcomings (Jenny et al. 1950, Vlamis et al. 1958, Christensen & Muller 1975). Substantial plant growth responses to phosphorus addition have shown the importance of this element in the Australian environment (Specht 1963, Groves 1965, Specht et al. 1977). Nevertheless this is not a general feature of all species in the region as Groves and Keratis (1976) have demonstrated that Australian Proteaceae die at high levels of nitrogen and phosphorus fertilization. In the Californian environment the eqivivocal importance of nitrogen and phosphorus has been shown by Hellmers et al. (1955), Schultz et al. (1958), McMaster et al. (1982) and Gray and Schlesinger (1983). They showed that N and P addition to soils caused varied growth responses, increased adult mortality and decreased seedling vigour in many of the characteristic species of the region. In fynbos ecosystems the only data available of nitrogen utilization patterns have been provided by Lewis and Stock (1978) who showed that members of the Proteaceae show small positive responses to nitrogen fertilization.

This study has concentrated on N cycling processes important in determining the structure and functioning of fynbos. It has been undertaken in parallel with a study of P cycling, because the role of these two elements in other mediterranen ecosystems is the most clearly understood of that of all the nutrients.

Clark (1981) has recently reviewed the evolutionary development of the nitrogen cycle concept which has evolved from the beginning of the current century. Initially, studies concentrated on individual N cycling process- or flow-orientated studies in agro-ecosystems (Löhnis 1913), mainly because of the economic importance of such systems, and it was not until the 1930's and 1940's that balance sheet studies were undertaken. Allison (1955) produced the first comprehensive review of such balance studies. The shift towards an ecosystem concept has been continued over the past 20 years with the development of compartmental and budgeted flow models (Bornmann & Likens 1967 Woodmansee 1978, Woodmansee et al. 1978, Whittaker et al. 1979, Gosz 1980, Herrera et al. 1981, Coleman et al. 1983). Finally, with the recent development and sophistication of computer technology more emphasis has been laid upon simulation models which can mimic the dynamic nature of biological and physical processes in complex ecosystems (Reuss & Innis 1977, van Keulen 1981, van Veen et al. 1981, Aber & Mellilo 1982, Tanji 1982, Pendleton et al. 1983). A similar pathway from initial studies of individual processes through to budget and simulation models is the research approach adopted in this project (although not sufficient detail has been attained to produce a complete budget flow model).

The use of N cycling studies to answer questions concerning the effect of management practices on system stability requires the estimation of the size of the soil and vegetation reserves of N, the input of N to the system from atmospheric and parent

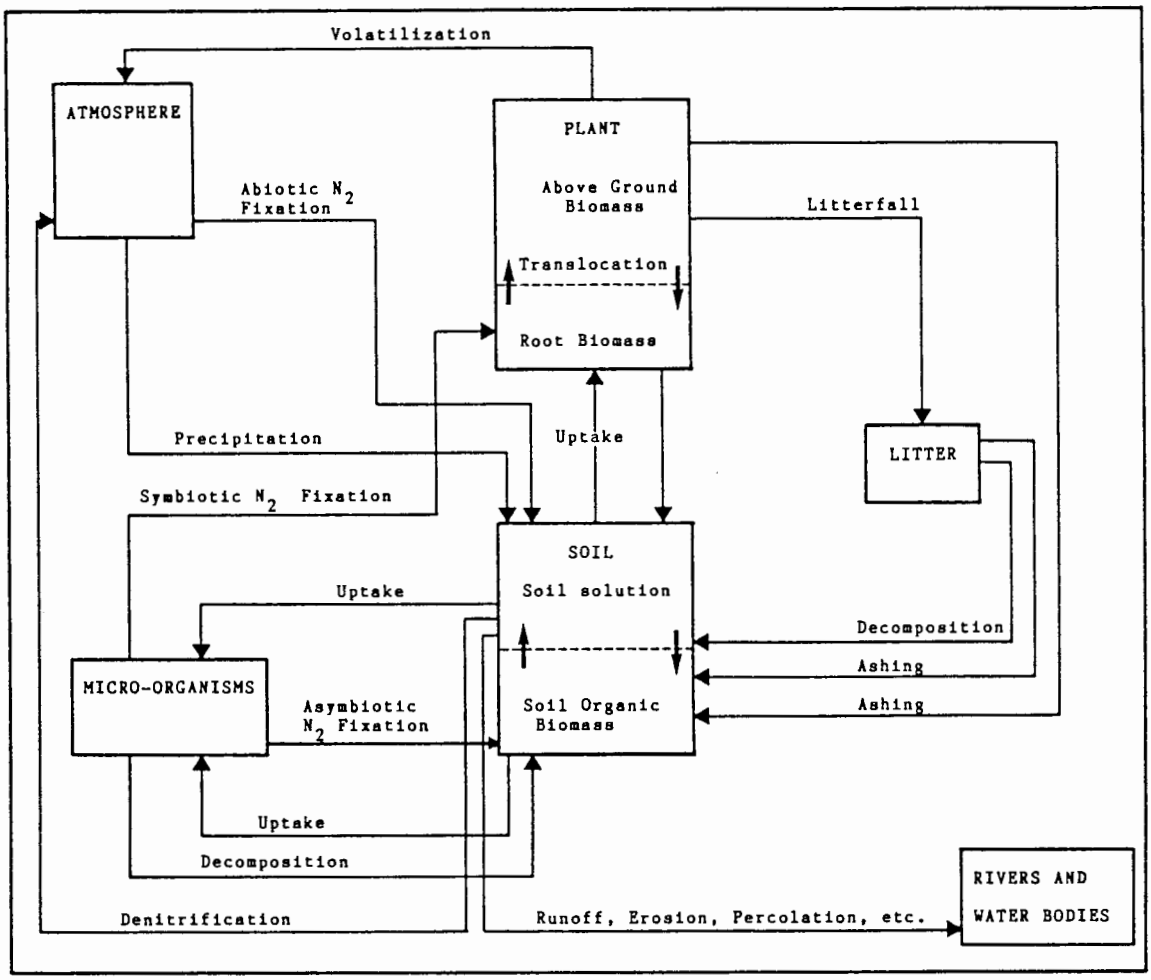


Figure 1.2: Major nitrogen compartments and potential pathways of nitrogen transfer in an ecosystem affected by fire (Adapted from Woodmansee & Wallach 1981, Rundel 1982).

material sources, and losses to drainage and the atmosphere. Nitrogen fluxes within and between compartments of the ecosystem (Fig. 1.2) also need to be calculated. An investigation of this type is a major undertaking and this project only explores areas of the N cycle in a coastal fynbos ecosystem which from research in other mediterranean ecosystems were thought to be of crucial importance in future management and stability of fynbos vegetation.

CHAPTER 2

STUDY AREA

The Pella Fynbos Intensive Study Site is located on the farm Burgherspost (33° 31'S : 18° 32'E) in the Malmesbury district of the Cape Province, South Africa (Fig. 2.1). The topography of the 269 ha site consists of gentle hills (altitude between 160-200m above sea level) with no major landforms or drainage systems.

The climate of the region, following the Köppen classification, is a true Mediterranean climate (Csa and Csb) which is characterised by dry summers and wet winters (Schulze 1947). The 40 year mean annual rainfall for the Burgherspost farm was 577 mm from 1928-1968 and the mean annual temperature at Philadelphia, a nearby weather station (33° 40'S : 18°35'E), was 17,3°C for the period from 1975-1980 (unpublished records from the South Africa Department of Agricultural and Technical Services, Winter Rainfall Region). As no wind data was collected at any of the weather stations surrounding the Pella site, the strength and direction of prevailing winds is uncertain. However, Fuggle and Ashton (1979), in a review of the climate of the Fynbos Biome, note that west of the north-south trend of the Hottentots Holland mountains, and their northern extension, summer winds are dominatly SE to S, while in winter winds from the NW and SW dominate.

The Pella site falls into the region of the South Western Cape which is occupied by an exceptionally

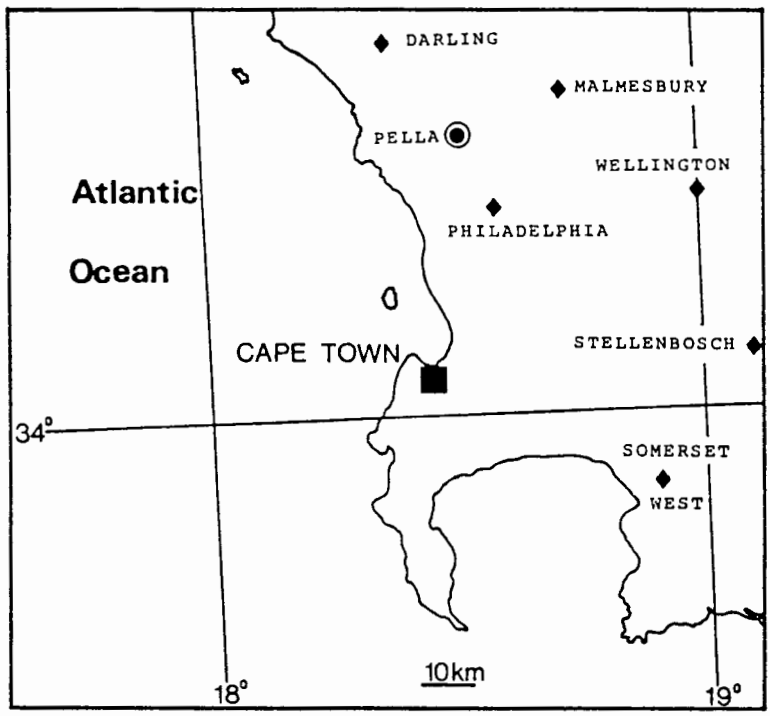


Figure 2.1: Location of the Pella Fynbos Biome Research site in the South Western Cape Province, South Africa.

species rich flora consisting of a mixture of heathland and sclerophyllous shrublands, known locally as fynbos. Taylor (1978) has identified fynbos as having three distinct physionomic groups present, namely the ubiquitous presence of the ericoid and restiod groups while the proteoid elements may not always be present. Kruger (1979) suggests that the only constant and differential floristic element of fynbos is the presence of the family Restionaceae.

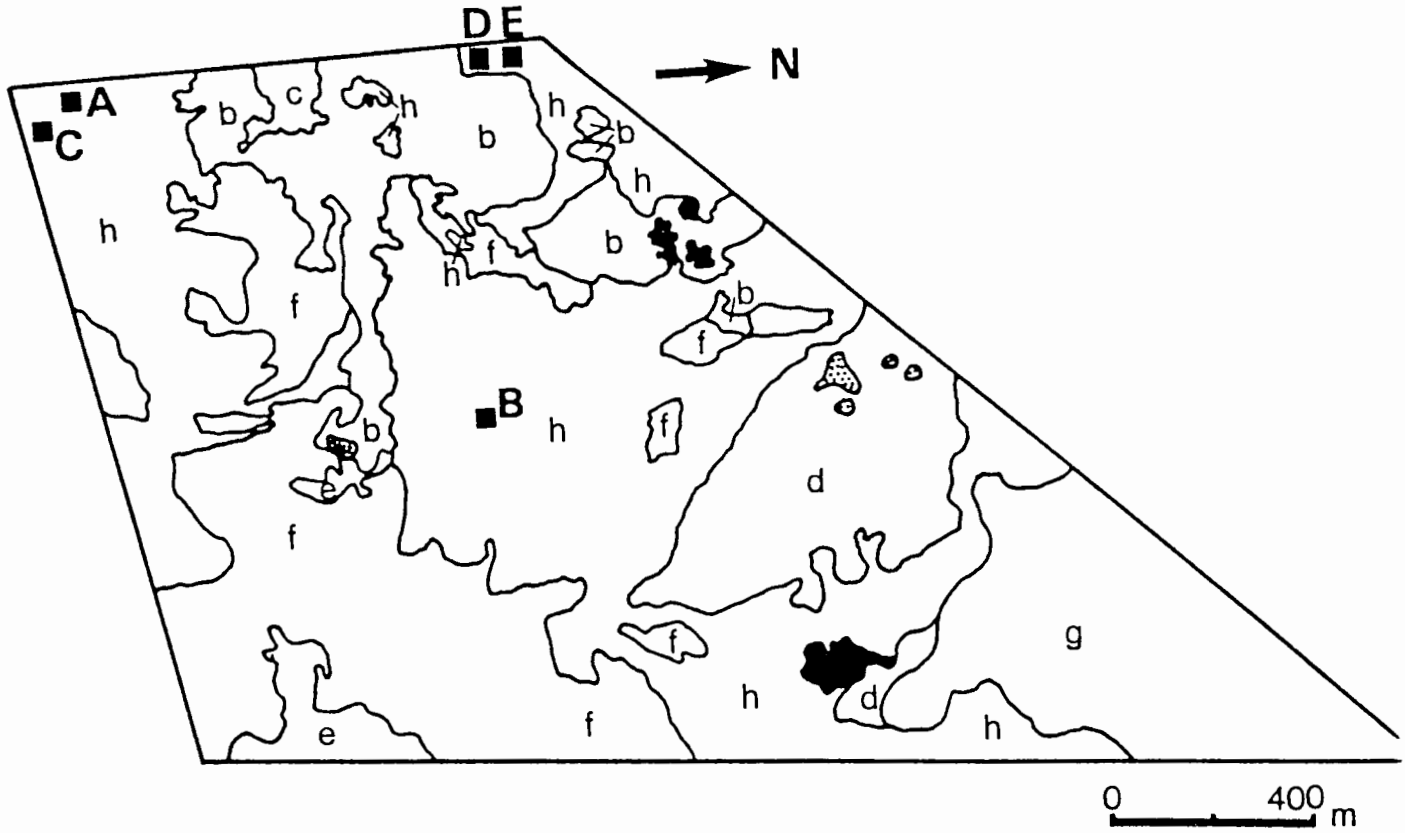
The vegetation of the study area is a component of Acock's (1953) veld type No. 47, Coastal Macchia (Fynbos), which occurs on limestone and sandy substrates of the western and southern coastal plains of the Cape Province. Moll et al. (1984) have reclassified the vegetation units of the Fynbos Biome and the Pella site may now be considered as Sand Plain Fynbos because it is a heathland which occurs on acidic leached sands. In addition to Sand Plain Fynbos, Moll et al. (1984) have recoginised two other sub-divisions of Acock's veld type, Coastal Fynbos, namely Elim Fynbos and Limestone Fynbos. These occur on laterite and limestone substrates respectively.

A phytosociological Braun-Blanquet vegetation

survey of the Pella site was undertaken by Boucher and Shepherd (pers comm.)* who found 8 different plant communities as shown in Figure 2.2. The N cycling research described in this project was undertaken in one community type of different ages, namely the Leucospermum parile - Thamnochortus punctatus Mid-high Open Shrubland (see study sites A-E in Fig. 2.2). The community is largely dominated by small-leaved shrubs: the ericoid element in the younger stages, while members of the Proteaceae dominate in older stands. The Restionaceae are always present. Important ericoid species at the site are Phylica cephalantha Sond., Phylica stipularis L., Passarina vulgaris Thoday and Greisebachia incana (Bartl.) Klotzsch. Restionaceae are represented by Thamnochortus punctatus Pill., Staberhoa distachya (Rottb.) Kunth and Cannomois accuminata (Thunb.) Pill.. Proteaceae evident in the older communities are Leucospermum parile L., Protea repens L. and Protea burchellii Stapf. The vegetation canopy height varied from 1-2m depending upon community age.

The soils are sandy, overlying granite bedrock to a depth of approximately 2m. The soils are aeolian in

* Boucher, C. & Shepherd, P., Botanical Research Institute, Stellenbosch, South Africa.



PLANT COMMUNITIES OF THE PELLA RESEARCH SITE

1. EUCLEA RACEMOSA Strandveld

1.1 SALVIA LANCEOLATA - WIBORGIA OBCORDATA Low to Mid-high Closed Shrubland

2. PHYLICA CEPHALANTHA Fynbos

B 2.1 DIOSPYROS GLABRA - PSAMMOTROPHA MYRIANTHA Mid-high Open Shrubland

C 2.2 DIOSPYROS GLABRA - ELATROPAPPUS RHINOCEROTIS Mid-high Sparse Shrubland

D 2.3 DIOSPYROS GLABRA - HERMANNEA ALNIFOLIA Mid-high Open Shrubland

E 2.4 DIASTELLA PROTEOIDES - BERZELIA ABROTANOIDES Mid-high Open Shrubland

F 2.5 DIASTELLA PROTEOIDES - ERODEA IMBRICATA Mid-high Sparse Shrubland

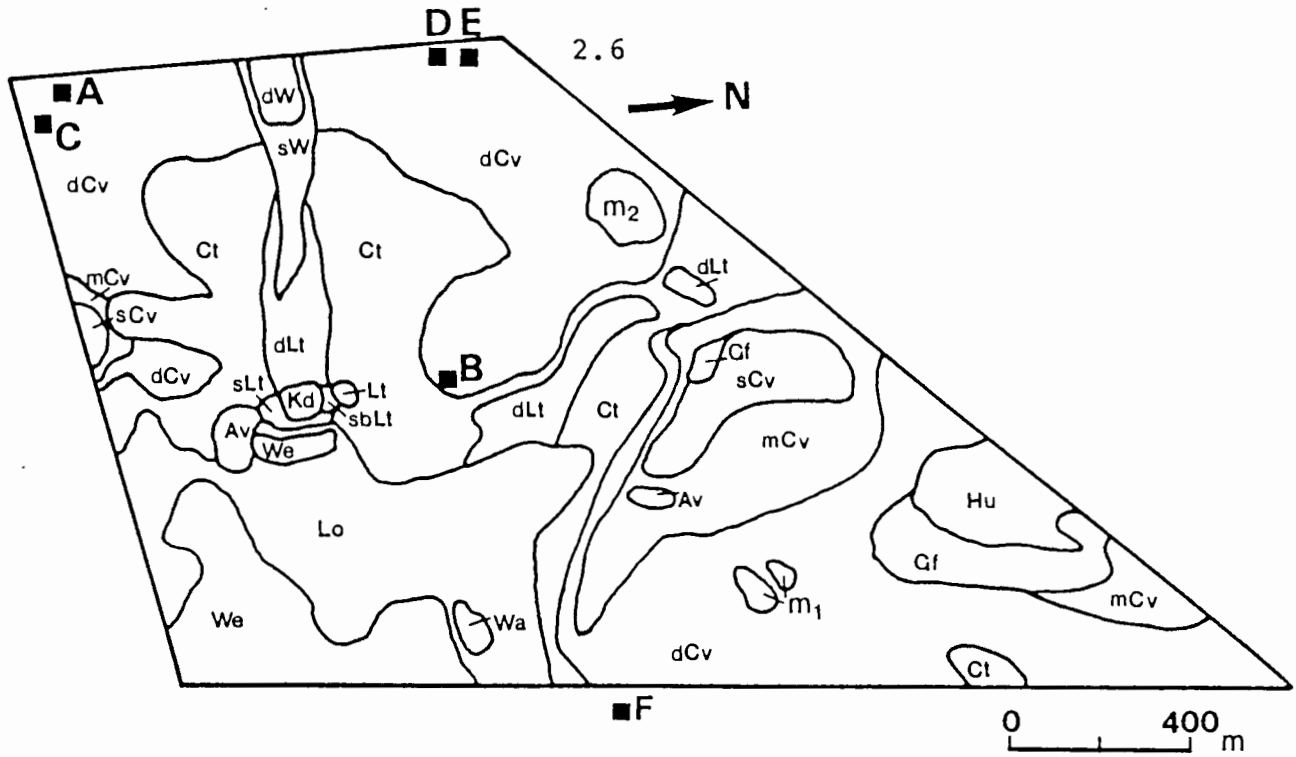
G 2.6 LEUCOSPERMUM PARILE - STOEBE LEUCOCEPHALA Low Mid-dense Shrubland

H 2.7 THAMNOCHORTUS PUNCTATUS - LEUCOSPERMUM PARILE Mid-high Open Shrubland

3. ALIEN COMMUNITIES

3.1 ACACIA SALIGNA Tall Closed Shrublands

Figure 2.2: Plant communities of the Pella site (after Boucher and Shepherd (in prep.)).



LAND TYPES

Dominant Feature	Subdivision Criteria	Map Symbol
Yellow sandy loam wash overlaying grey medium sands		
Gritty material badly sorted with a number of thick (2,5cm) clay lamellae	Wash overburden less than 50cm in depth and less than 3 clay lamellae	sW
	Wash overburden greater than 50cm in depth, and greater than 3 clay lamellae	dW
Mounds of darkish yellow-brown medium sands		
Mounds with darker colours and greater particulate organic matter than surrounding soils	Large isolated mounds	M_1
	Group of smaller mounds occurring together in dry soils	M_2

SOIL FORMS AT PELLA (South African Binomial System 1977)

Hutton	Hu	Lamotte	Lt
Griffin	Gf	Constantia	Ct
Clovelly	Cv	Kroonstad	Kd
Avalon	Av	Wasbank	Wa
Longlands	Lo	Westleigh	We

SOIL CONSOCIATIONS

Dominant feature	Soil classification: Form	Series	Phase	Map symbol
Well drained red and yellow medium sands				
Saprolite within 2m				
Uniform red	Hu	11(21)	Saprolite 0-2m	Hu
Uniform yellow over red	Gf	10	Saprolite 0-2m	Gf
Uniform yellow	Cv	11	Saprolite 0-1m	sCv
			Saprolite 1-2m	mCv
No saprolite within 2m				
Uniform yellow	Cv	11		dCv
Moderately drained yellow medium sands				
Uniform yellow / soft plinthic	Av	12	Saprolite 0-2m	Av
Moderate to poorly drained grey medium sands on gleyed clay				
Bleached E over gleyed clay	Kd	11	Saprolite 1-2m	Kd
Moderate to poorly drained grey medium sands				
Soft plinthic mottling below bleached E	Lo	20		Lo
Hard plinthic layer below bleached E	We	20	Plinthic layer depth 1m	Wa
Soft plinthic mottling	We	20		We
Podzolized grey medium sands with bleached E horizon				
Saprolite within 2m				
Dark brown ferrihumic B below E	Lt	11	Friable ferrihumic	sLt
			Hard ferrihumic	sbLt
No saprolite within 2m				
Dark brown ferrihumic B below E			Friable ferrihumic	dLt
Yellow brown sands below E	Ct	11	Ferrihumic nodules	Ct

Figure 2.3: Soil forms, land types and soil consociations at the Pella site (after Fry and Lambrechts (in prep.)).

origin derived from a mixture of weathered Table Mountain Sandstone and granite geological formations, and are thought to have been deposited approximately 10,000 years ago (Fry & Lambrechts pers comm.)*. Following the South African Binomial soil classification system (MacVicar et al. 1977) a number of soil forms have been recognised in a survey of the Pella site (Fig. 2.3, Fry & Lambrechts pers comm.)*. These forms include freely draining soils forming a catena of Hutton, Griffin and Clovelly soil forms with moderately poorly drained sands at the base of the slope (Westleigh and Longlands soil forms). The nitrogen studies reported in this project concentrated on one soil form, namely the Clovelly soil form, which is recognised by an Orthic A horizon over a yellow-brown apedal B horizon (MacVicar et al. 1977) (see study sites A - F in Fig. 2.3). The soils at Pella are considered to be of a low nutrient status and Mitchell et al. (1984) have shown the Clovelly soil to have a low phosphorous status and low pH values (pH and ranges from 3,68 - 4,30 at the soil surface).

* Fry, M. & Lambrechts, J.J.N., Department of Soil Science, University of Stellenbosch, Stellenbosch, South Africa.

Historical landuse studies were undertaken at Pella, and Brownlie (1983) has shown fire to have been the major site perturbation subsequent to 1920. Other forms of disturbance, namely grazing, browsing and bushcutting, appear to have had a minimal impact on the site. In Figures 2.4, 2.5 and 2.6 the impact of repeated fires on vegetation cover of the sample sites may be seen. The November 1980 fire was a moderate to intense fire which destroyed the oldest stands of vegetation on the Pella site. All fire studies reported in this project were related to this wildfire.

It is thus apparent that the Fynbos Biome Intensive Study at Pella offers a mosaic of different ages of similar vegetation occurring on the same soil forms. This provides an ideal area for ecophysiological research into the nitrogen cycling processes important in coastal fynbos vegetation.

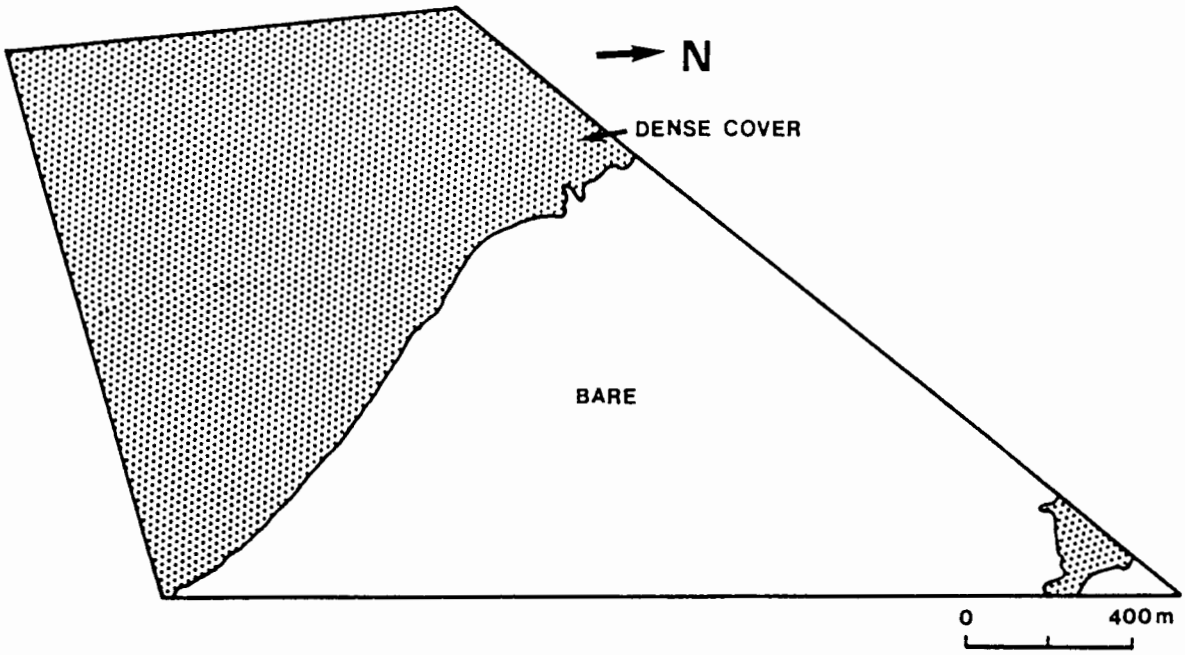


Figure 2.4: Vegetation cover of the Pella site during 1966 after a wildfire (Brownlie 1983).

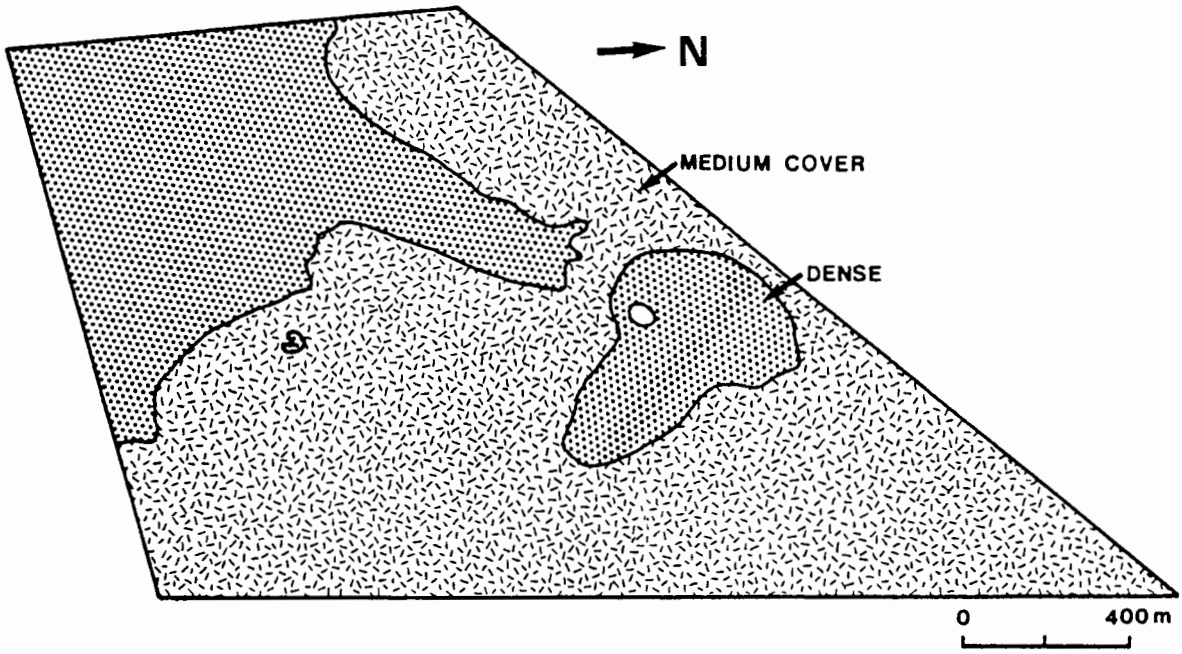


Figure 2.5: Vegetation cover of the Pella site during 1979 after a wildfire in 1976 (Brownlie 1983).

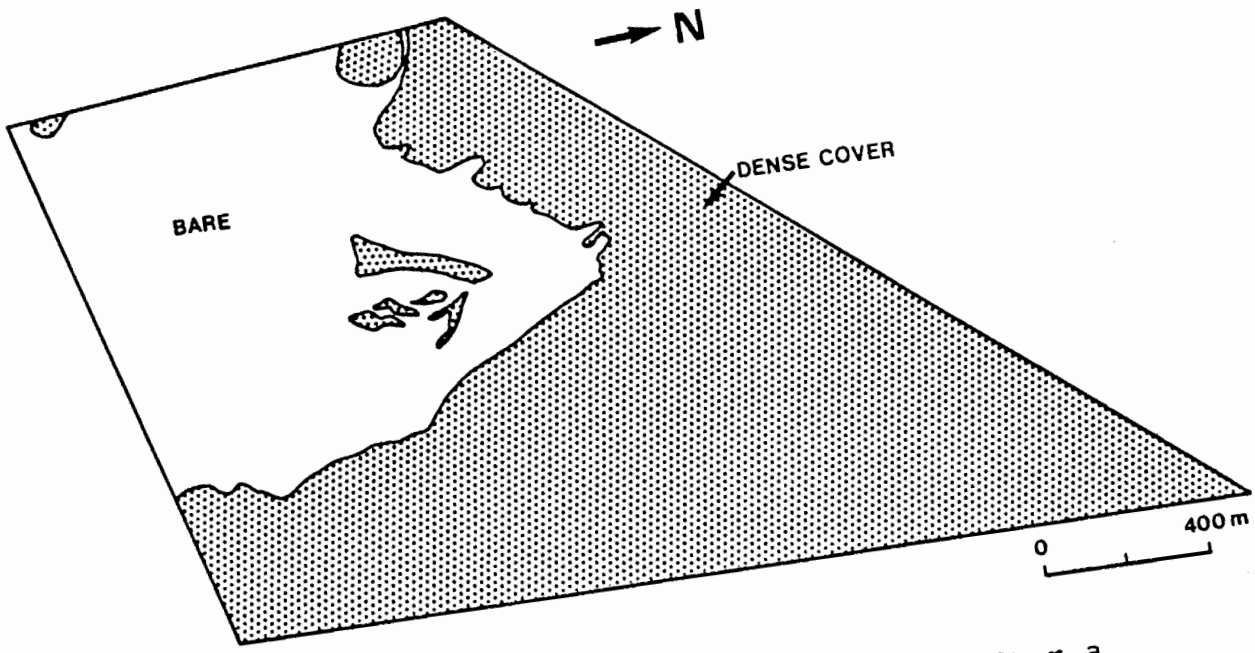


Figure 2.6: Vegetation cover of the Pella site after a wildfire in 1980 (Brownlie 1983).

CHAPTER 3

METHODS AND MATERIALS

Chapter includes two published papers:

An evaluation of some manual colorimetric methods for the determination of inorganic nitrogen in soil extracts. Communications in Soil Science and Plant Analysis. 14 (10) : 925 - 936 (1983).

- Section 3.1.5

Extraction of nitrate reductase from members of the South African Proteaceae. South African Journal of Botany 1 (4) : 124 - 126 (1982).

- Section 3.2.4

Contents

3.2.3 Determination of total N in plant material.

3.2.4 Extraction of nitrate reductase from members of the South African Proteaceae.

3.2.5 Design of ^{15}N feeding experiments to determine plant N uptake and assimilation.

3.3 Atmospheric deposition collection and bulk precipitation nitrogen analytical procedures.

3.3.1 Bulk precipitation collection.

3.3.2 Determination of nitrate in bulk precipitation.

3.3.3 Determination of ammonium in bulk precipitation.

3.3.4 Determination of total N in bulk precipitation.

3.1 Soil collection and soil nitrogen analytical procedures.

3.1.1 Soil sample collection.

Soil samples were collected for the investigation of spatial and temporal changes in soil N on a monthly basis from October 1979 until November 1981. The sample site at the Pella Research Station (site A in Fig. 2.3) was 2 ha in extent and consisted of a coastal fynbos community growing on a Clovelly soil form (Boucher & Fry in prep.). Each month, sampling sites were chosen within the 2 ha site by locating undisturbed areas forming open spaces between individual plants of 2 or more of the following species:- Phylica cephalantha, Staberhoa distachya, Leucospermum parile, Thamnochortus punctatus or Protea repens. Pits of surface area 1m x 1m and 1,2m in depth were dug every 2 months and soil samples removed from the four faces of the pit in the following depth categories: 1 to 2,5cm, 2,5 to 7,5cm, 7,5 to 12,5, 12,5 to 17,5, 17,5 to 22,5cm, 27,5 to 32,5cm, 37,5 to 42,5cm, 67,5 to 72,5cm and 97,5 to 102,5cm. The depth categories are referred to by their depth midpoints except for the surface 2,5cm which is called 0 (0, 5, 10, 15, 20, 30, 40, 70, 100). Soil samples collected for the intermediate months were from pits 30cm deep with 5 depth categories between 0 and 22,5cm (depth midpoints

were 0, 5, 10, 15, 20).

To obviate possible complications caused by diurnal changes in soil N form and content, samples were always collected between 09 00 and 11 00 hours. The soil samples were transported to the laboratory on the same day as collection and extracted immediately, the extract being used for the determination of soil inorganic N. Moisture content, organic matter content, total N levels and soil pH were also determined (see Sections 3.1.6 to 3.1.9). Sample pits were refilled and identified in the field by inserting markers to prevent resampling of the same area.

3.1.2 Collection of soil from rhizosphere regions of selected plants.

The spatial distribution of organic and inorganic N in soil of the rhizosphere region of selected fynbos plants growing on a Clovelly soil form (site A Fig. 2.3) was investigated during November 1979. Six cores of 6,7cm in diameter and 10cm in depth were collected in the open and under canopies of the following plants: L. parile, S. distachya, P. repens and P. cephalantha. The soils were transported to the laboratory where they were extracted and analysed for nitrate, nitrite, ammonium and total N as well as for organic matter, pH and moisture content.

3.1.3 Soil preparation for N analysis.

3.1.3.1 Soil preparation for inorganic N extraction.

Analysis of soil samples for available nutrients, particularly nitrogen, are influenced to varying degrees by drying, grinding, storage, sieving and other preparative treatments. It has been shown that air-drying can increase the quantity of mineral N as well as alter the forms in which it is present. Barker (1974) reported little change in nitrate level with air-drying but that nitrite and ammonium levels may decrease. On the other hand Gasser (1961) has shown increases in both NH_4-N and NO_3-N contents of the soil upon air-drying with the ammonium level changing to a greater extent. Other workers (Allen & Grimshaw 1962, Breimer & Slangen 1981) have shown that low temperature storage and rapid high temperature drying also affect mineral N quantity and form. They recommend the extraction of only fresh field-moist samples to exclude the possible transformation of one form of nitrogen into another before analysis. Sieving and grinding may also cause changes in mineral N but the extent of these changes has not been fully studied (Lockman 1980).

3.1.3.2 Soil preparation for total N determinations.

Soil samples for the determination of total nitrogen were air-dried in the laboratory. Hesse (1971) reports that air-drying a soil before total N determination has little affect on the total N content and as modifications to the Kjeldahl procedure enable it to estimate all forms of mineral N (Bremner 1965a, 1965d) any transformations from one N form to another during the drying process does not influence the determination.

3.1.4 Soil inorganic N extraction.

The choice of chemical extractants used in the preparation of soil extracts for the determination of inorganic N are determined by a number of requirements viz:

a) quantitative extraction of the inorganic N form being studied,
b) the extractant must not impair the sensitivity of the methods available for the determination of the N form being studied,
c) the extracting solution should prevent biologically or chemically induced changes in the quantity or quality of N present in the sample,
d) if possible a single extractant should be adopted for the simultaneous extraction of all soluble N forms, and

e) if possible the extract should require little or no decoloration before colorimetric analysis.

Bremner and Shaw (1958), Bremner (1965a) and Sahrawat (1979) have undertaken evaluations of a number of extracting solutions and Sahrawat (1979) has shown the efficiency of the solutions in extracting exchangeable NH_4-N to follow the sequence, KCl > NaCl,pH 2,5 > NaCl > CH_3COONa, pH 3,0 > Morgan's reagent. Bremner (1965a) reported that 1M or 2M KCl soil extracts were satisfactory for quantitive extraction of exchangeable ammonium and that recovery of nitrate and nitrite was not affected. In addition he found that KCl extracts prevented chemical and biological changes in N quantity or quality from occurring.

The sandy, low clay content Clovelly soil form studied in this investigation required no decoloration and the 1M KCl extracts were clearer than water extracts of the same samples. The following procedure was used for the simultaneous extraction of exchangeable ammonium, nitrite and nitrate: Ten grams of fresh soil were added to 40 ml of 1M KCl and shaken for 1 hour. The extract was filtered through Whatman No. 1 filter paper and the filtrate analysed for nitrate, nitrite and ammonium. Blanks were prepared by filtering 40 ml 1M KCl through filter paper and the filtrate analysed for nitrate, nitrite and ammonium which might be present as a contaminant in the filter paper.

3.1.5 An evaluation of some manual colorimetric methods for determination of inorganic nitrogen in soil extracts.

W.D. Stock. Botany Department, University of Cape Town, Rondebosch, Cape, 7700, South Africa.

A wide range of methods are available for the determination of nitrate, nitrite and ammonium in soil extracts. An investigation of methods most suited to the aims of this project was undertaken to find techniques which were rapid, versatile, precise and relatively inexpensive. The evaluation of a number of colorimetric methods available was undertaken and the results are reported in this section which was published as a paper in Communications in Soil Science and Plant Analysis 14 (10): 925-936 (1983).

INTRODUCTION

Potentiometric, colorimetric and distillation methods for the determination of soil nitrate, nitrite, and exchangeable ammonium are all widely used in soil and water analytical laboratories and the limitations imposed by the expense, rapidity, versatility and precision of the individual methods are the deciding factors in selecting which methods are to be used in a particular laboratory (Jackson 1958, Henriksen & Selmer-Olsen 1970, Allen et al. 1974, Siegel 1980). A large range of soil inorganic N extractants are available (Bremner 1965a, Pedrazzini et al. 1979, Sahrawat 1979) and the adoption of a single solution for the simultaneous extraction and later analysis of all inorganic N forms would greatly accelerate and simplify the task of routine N extraction and analysis.

Bremner (1965a) and Sahrawat (1979) have reported 1M or 2M KCl solutions to be the most effective NH_4-N extractants, whilst NO_2-N and NO_3-N recovery does not appear to be greatly affected by this extractant. A further advantage of the KCl extract is that it may be stored for a number of days before analysis as no changes in NH_4-N, NO_3-N or NO_2-N levels have been observed (Bremner 1965a).

As distillation procedures are time consuming and potentiometric methods (notably the nitrate electrode)

are prone to Cl^- ion interference (Orion Nitrate Electrode Manual) a number of manual colorimetric methods have been investigated to find methods suitable for large numbers of N determinations in a laboratory not equipped with an AutoAnalyser.

In this investigation recently described methods for nitrate analysis, i.e. Szechrome NAS reagent (Szechrome NAS reagent pamphlet), chromotropic acid (CTA) (Sims & Jackson 1971) and a copperised cadmium reduction technique (Cu/Cd) (Wood et al. 1967, Henriksen & Selmer-Olsen 1970, Bate & Heelas 1975, Huffman & Barbarick 1981) have been assessed in relation to possible Cl^- ion interference as these methods are reported to be less sensitive to Cl^- than the phenoldisulphonic acid method (PDA). Nitrite concentration was estimated by the Griess-Ilosvay method of diazotization with sulphanilamide and then coupled with N-(1-napthyl) ethylenediamine to form an azo dye (Jackson 1958, Allen et al. 1974). Bremner and Shaw (1958) have shown Shinn's modification (1941) of this technique to be satisfactory for nitrite determination in 1M KCl and 1M K_2SO_4 soil extracts. Ammonium was determined by a manual indophenol blue method (IPB, Berthelot's reaction) which is extremely sensitive but requires stringent control to maintain reproducibility when applied as a manual method (Patton & Couch 1977).

Results obtained from a range of samples analysed using manual colorimetric methods for nitrate and ammonium determinations in 1M KCl extracts were compared with results from automatic analysis to show that the reliability and accuracy obtainable with manual methods is comparable with that obtained from the more expensive and sophisticated automatic analytical procedures.

MATERIALS AND METHODS

Soil Inorganic Nitrogen Extraction.

Ten grams of fresh soil were added to 40 ml of 1M KCl and shaken for 1 h. The solution was filtered through Whatman No. 1 filter paper and the filtrate analysed for nitrate, nitrite and ammonium. Blanks were prepared by filtering 40 ml 1M KCl through filter paper and analysing the solutions for ammonium which is normally present on the filter paper.

Nitrate Determinations

a) Chromotropic Acid Method

To 3 ml of sample prepared in distilled water or 1M KCl 7 ml of 0,01% chromotropic acid working solution were added (prepared as described by Sims & Jackson 1971). The colour development of the CTA-NO_3 complex was read on a spectrophotometer after 10 min at 430nm. Unknown

concentrations were calculated from a standard curve constructed for nitrate values between 0,3 -8,0 µg N ml^{-1}.

b) Szechrome NAS Reagent

To a 0,5 ml sample 2,5 ml of Szechrome NAS reagent were added. The mixture was agitated whereupon colour development was read at 570nm after 5 min. The colour is reported to be stable for 1 h (Szechrome NAS reagent pamphlet). Nitrate concentrations in the soil extracts were determined from a standard curve constructed between 0,1 and 2,0 µg N ml^{-1}. Szechrome NAS reagent was prepared by adding 5g Szechrome NAS powder to 1 1000 ml of a 1 : 1 concentrated sulphuric acid phosphoric acid mixture.

c) Copperised Cadmium Reduction Analysis

Nitrate reduction was performed by adding 2 g wet weight of prepared Cu/Cd to 3 ml of soil extract plus 0,1 ml 1M MgCl (to overcome any possible phosphate interference) and 1,9 ml of a 0,4M NH_4Cl buffer adjusted to pH 9,6 with NH_4OH. The mixture was shaken for precisely 10 min whereupon a 1 ml aliquot was removed and NO_2-N determined by the Griess-Ilosvay method (Bate & Heelas 1975). Batches of 15 samples, 3 blanks and 5 standards were run simultaneously. Nitrate concentrations were calculated from a standard curve constructed between 0,1 - 1,5 µg N ml^{-1}.

d) Automatic Analysis

Nitrate was determined in 1M KCl soil extracts with a Technicon AutoAnalyser utilising a copperised cadmium reduction column as described by Grasshoff (1976).

Nitrite Determination

Nitrite was determined in the 1M KCl extracts by the Griess-Ilosvay method in which 1 ml of 1% (w/v) sulphanilamide in 1,5M HCl and 1 ml of 0,01% (w/v) N-(1-napthyl) ethylenediamine HCl solution were added to 1 ml of the soil extract. Colour was allowed to develop for 10 min after which the absorbance was read at 540 nm (Allen et al. 1974). NO_2-N standards were prepared in the range from 0,1 - 1,0 µg N ml^{-1}.

Ammonium Determination

a) Manual Indo-Phenol Blue Determination.

To 2 ml of sample or standard the following reagents were sequentially added with Gilson Pipetman dispensors: a) 1,6 ml 10% (w/v) sodium potassium tartrate solution, b) 0,2 ml 0,16% (w/v) sodium nitroprusside solution, c) 0,4 ml sodium phenate reagent prepared fresh each day by dissolving 50 g phenol in 250 ml 40% NaOH and diluting to 400 ml, and d) 0,2 ml sodium hypochlorite with 5% available Cl^-. The reagents were mixed and the solution made up to 10 ml. After 20 min incubation in a waterbath

at 40°C the solutions were cooled and the absorbance read within 10 min at 625nm. Batches of 15 samples, 3 blanks and 5 standards were run simultaneously. Standards were prepared in the range 0,1 - 3,0 µg N ml^{-1}. The timing of all stages of reagent addition and incubation are critical for reproducible colour development (Allen et al. 1974).

b) Automatic Analysis.

Ammonia was determined in 1M KCl extracts with a Technicon AutoAnalyser using the indo-phenol blue method (Strickland & Parsons 1972, Grasshoff 1976).

RESULTS AND DISCUSSION

In Figures 3.1 and 3.2 it is evident that the CTA and Szechrome NAS methods suffer interference from the high Cl^- levels present in the 1M KCl extracts. The sensitivity of both techniques is drastically reduced when assaying standards prepared in 1M KCl compared with the same standards prepared in distilled water. The Szechrome NAS method appears to be less severely affected than the CTA method (Szechrome NAS standard curve is still linear, although the slope is reduced with a correlation coefficient r = 0,993).

Sims & Jackson (1971) investigated Cl^- interference of the CTA method by the addition of Cl^- in

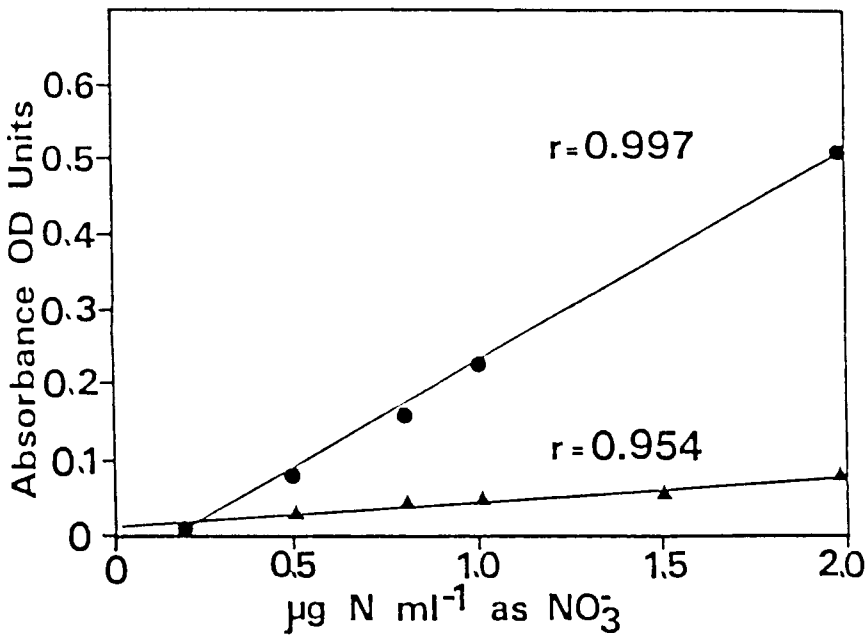


Figure 3.1: Comparison of standard curves produced by colorimetric analysis of NO_3^--N the chromotropic acid method when the standards are prepared in distilled water (●—●) and 1M KCL (▲—▲).

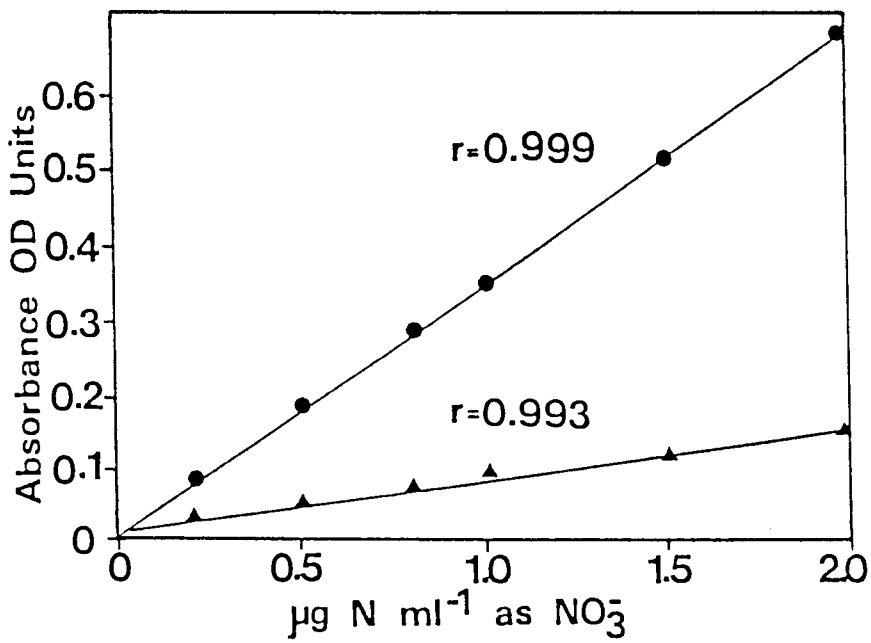


Figure 3.2: Comparison of standard curves produced by colorimetric analysis of NO_3^--N using Szechrome analytical reagent when the standards are prepared in distilled water (●—●) and 1M KCL (▲—▲).

concentrations of up to 10 μg ml^{-1} and found no significant interference, but in 1M KCl solutions the Cl^- concentration greatly exceeds that tested by Sims and Jackson (1971) and colour development is inhibited. The Szechrome NAS reagent is reported to be interference free at Cl^- concentrations below 1000 μg ml^{-1} (Szechrome NAS reagent pamphlet). The Cl^- concentration in the extracting solution (35453 μg ml^{-1} Cl^{-1}) exceeds this upper limit and interference is apparent (Fig. 3.2). These two simple and rapid colorimetric methods are not suitable for nitrate determination in 1M KCl soil extracts.

In Table 3.1 colour development of NO_3-N and NH_4-N standards prepared in 1M KCl and determined by manual Cu/Cd reduction and Indo-Phenol Blue methods respectively are shown. Both techniques suffer little interference from Cl^- and the standard curve constructed for each is linear with a coefficient of determination for the linear regression of the standard curves greater than r = 0,995 in both cases. These methods have been utilised for inorganic N determinations in sea water and no Cl^- interference has been reported (Woods 1967, Henricksen & Selmer-Olsen 1970).

TABLE 3.1: Absorbance values obtained for the construction of standard curves for manual Cu/Cd reduction determination of NO_3-N and indo-phenol blue determination of NH_4-N. Each absorbance value is the mean of 4 determinations for that concentration and the coefficient of variation (%CV) for each concentration is shown as well as the linear regression of N concentration versus absorbance.

Standard µg N ml^{-1}	Copper / Cadmium OD Units		Indo-Phenol Blue OD Units	
	Mean	% CV	Mean	% CV
0,1	0,063	4,2	-	-
0,2	0,131	2,7	0,023	11,5
0,5	0,317	0,7	0,082	2,7
0,8	0,503	1,4	-	-
1,0	0,626	1,3	0,215	1,9
2,0	-	-	0,460	1,3
3,0	-	-	0,674	1,6
	y = 0,004 + 0,623x		y = -0,026 + 0,236x	
	r = 1,000		r = 0,999	

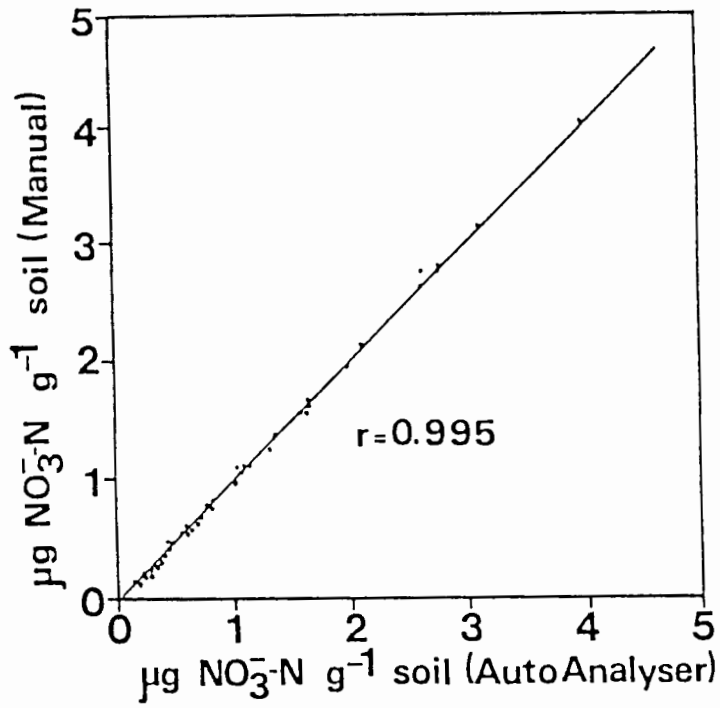


Figure 3.3: Relationship between nitrate nitrogen concentration obtained in soil extracts when determined by manual and automatic copperized cadmium reduction techniques (n = 40).

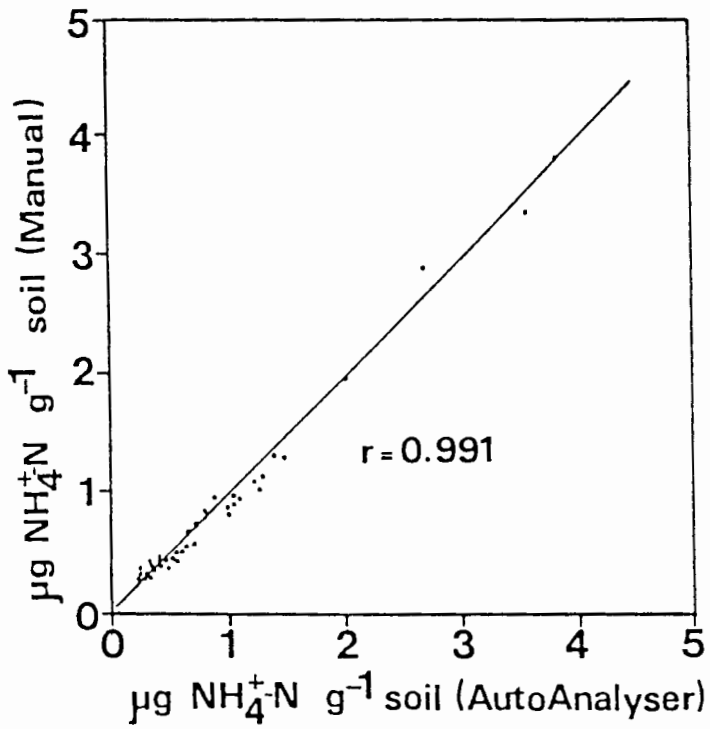


Figure 3.4: Relationship between ammonium nitrogen concentration obtained in soil extracts when determined by manual and automatic indo-phenol blue procedures (n = 40).

Manual colorimetric methods of inorganic N determination were compared with Auto Analyser N determinations of the same samples to investigate the accuracy and reliability of these methods. It is apparent from Figures 3.3 and 3.4 that the results obtained from manual and automatic procedures are in close agreement for both nitrate and ammonia determinations. In both cases the correlation coefficient for the linear regression of manual versus automatic nitrate and ammonium determinations was greater than 0,990.

Manual methods were performed at a rate of 60 - 80 nitrate or ammonium samples per person per day which enabled large numbers of samples to be analysed in a laboratory without the use of an Auto Analyser.

ACKNOWLEDGEMENTS

The author would like to thank Dr. P. Bartlett and Mrs. C. Weimar of the National Research Institute for Oceanology for undertaking the analysis of nitrate and ammonia on the Technikon Auto Analyser. I would also like to thank Professor O.A.M. Lewis for comments concerning the preparation of the manuscript. This work was funded by the Council for Scientific and Industrial Research as part of the Fynbos Biome Project.

3.1.6 Determination of total nitrogen in soil.

Methods available for the determination of organic N and other complex materials containing several forms of nitrogen are the Dumas method (dry oxidation) and the Kjeldahl method (wet oxidation) (Jackson 1958, Hesse 1971, Avery et al. 1974, Nelson & Sommers 1980). Bremner (1960) has shown that the Kjeldahl method is satisfactory for N determinations in most soils because there are few naturally occurring forms of combined N which cannot be determined satisfactorily by the procedure, or modifications of it (such as the use of salicylic acid and sodium thiosulphate to include the assay of nitrate and nitrite).

The following micro-Kjeldahl digestion procedure with modifications to include nitrate estimation (Bremner 1965d) has been adopted in this project as standard practice for all soil and plant analyses.

3.1.6.1 Micro-Kjeldahl digestion of total N in soils.

A 1,00 or 2,00 g air-dried 2 mm sieved soil sample was placed in a 75 ml thick walled digestion tube and moistened with 1 ml of double-deionized water. After 30 min 3 ml concentrated nitrogen-free AR H_2SO_4 (specific gravity 1,84) containing 34 g l^{-1} salicylic acid was

added together with a selenium catalyst tablet (BDH, Poole, U.K. containing 0,05 g of selenium and 1 g of sodium sulphate) and followed by 0,2 g sodium thiosulphate. The salicylic acid and sodium thiosulphate were added to quantitatively convert all NO_3-N to NH_4-N in the digest (Bremner 1965d). Blank determinations were made on the above reagents in the absence of soil.

The tubes were placed in an aluminium heating block at 150°C for 1 hour to evaporate off the water without the sample frothing (Nelson & Sommers 1972, 1973). The temperature was then increased in stages to 375°C over the next 3 hours. When the samples had cleared after the initial charring, the samples were allowed to digest for 2 hours. The digest was cooled and made up to a final volume of 50 ml before NH_4-N analysis, either by the distillation and titration technique or by the colorimetric procedure described below.

3.1.6.2 Distillation and titration determination of ammonium in Kjeldahl digests.

A Markham micro-distillation still was used to distil over the ammonia from a 20 ml aliquot of the Kjeldahl digest after alkalisation with 12 ml 50% (w/v) NaOH. The distillate (30 ml) was collected in 2 ml 0,02M HCl containing a screened methyl red indicator (Tashiro's indicator). The distillate was titrated

against 0,005M NaOH until the grey-coloured end point was reached. The nitrogen content was calculated from the titration value where 1 ml 0,005M NaOH = 70 µg N.

3.1.6.3 Colorimetric determination of NH_4-N in Kjeldahl digests.

The colorimetric indo-phenol blue method for the determination of ammonium-nitrogen was first reported by Berthelot more than a century ago (Berthelot 1859). The method has been slow to supplant distillation and titration because of its irreproducible colour development (Patton & Crouch 1977). It is further complicated by interference from salts in the Kjeldahl catalyst mixtures (Hg,Cu) and by differences in acidity between digest solutions (Harwood & Huyser 1970, Smith 1980, Ngo et al. 1981). Other factors important in reproducible colour development are the presence or absence of coupling reagents ("catalysts"), strict temperature control during colour formation and precise timing of colour development. (Tetlow & Wilson 1964, Allen et al. 1974, Smith 1980, Nkonge & Ballance 1982).

The method adopted is a modification of that described by Allen et al. (1974) in which nitroprusside is utilised as the coupling agent and selenium is used in preference to mercury as a Kjeldahl catalyst to avoid

interference from mercury.

The method is as follows: To a 1 ml Kjeldahl digest sample the following reagents were added sequentially with Gilson Pipetman dispensers:

a) 1,6 ml 10% (w/v) sodium potassium tartrate solution,

b) 0,2 ml 0,16% (w/v) sodium nitroprusside solution,

c) 0,8 ml sodium phenate reagent prepared fresh each day by dissolving 50 g phenol in 250 ml 40% (w/v) NaOH and diluting to 400 ml, and

d) 0,2 ml sodium hypochlorite with 5% available Cl^-.

The reagents were mixed and the solution made up to 10 ml. After incubating for 20 min in a waterbath at 40°C the solutions were cooled and the absorbance read within 10 min at 625nm. Batches of 15 samples, 3 blanks and 5 standards were run simultaneously. Standards were prepared by performing Kjeldahl digestions of known quantities of NH_4Cl and diluting the digests as described above for the samples.

The indo-phenol blue method was compared to the distillation-titration technique before it was adopted as a standard technique. A range of soils with different N contents were chosen. Kjeldahl digestions were undertaken using selenium catalyst tablets, and the digests made up to 50 ml. The digests were analysed for ammonium using both the distillation-titration and the

indo-phenol blue procedures. In Figure 3.5 the linear relationship between the two methods is shown and the correlation coefficient of the regression was found to be r = 0,97. In view of this good correlation between the two methods and the time saving achieved by the use of the indo-phenol blue method, it was decided to use this procedure for the estimation of the NH_4-N in Kjeldahl digests.

3.1.7 Determination of soil moisture content.

Moisture content of soils was determined gravimetrically by adding approximately 8 g of fresh soil to a preweighed dry crucible. This was weighed and placed in a forced draught oven at 105°C for 24 hours. After cooling in a desiccator the loss in mass was determined and expressed as a percentage of dry mass.

3.1.8 Determination of soil organic matter and total organic carbon.

Soil organic matter may be determined by mass loss, by wet combustion or by dry combustion. For routine determinations of non-calcareous soils, such as those encountered in this study, the mass loss determination gives an estimate of organic matter (Hesse 1971). After

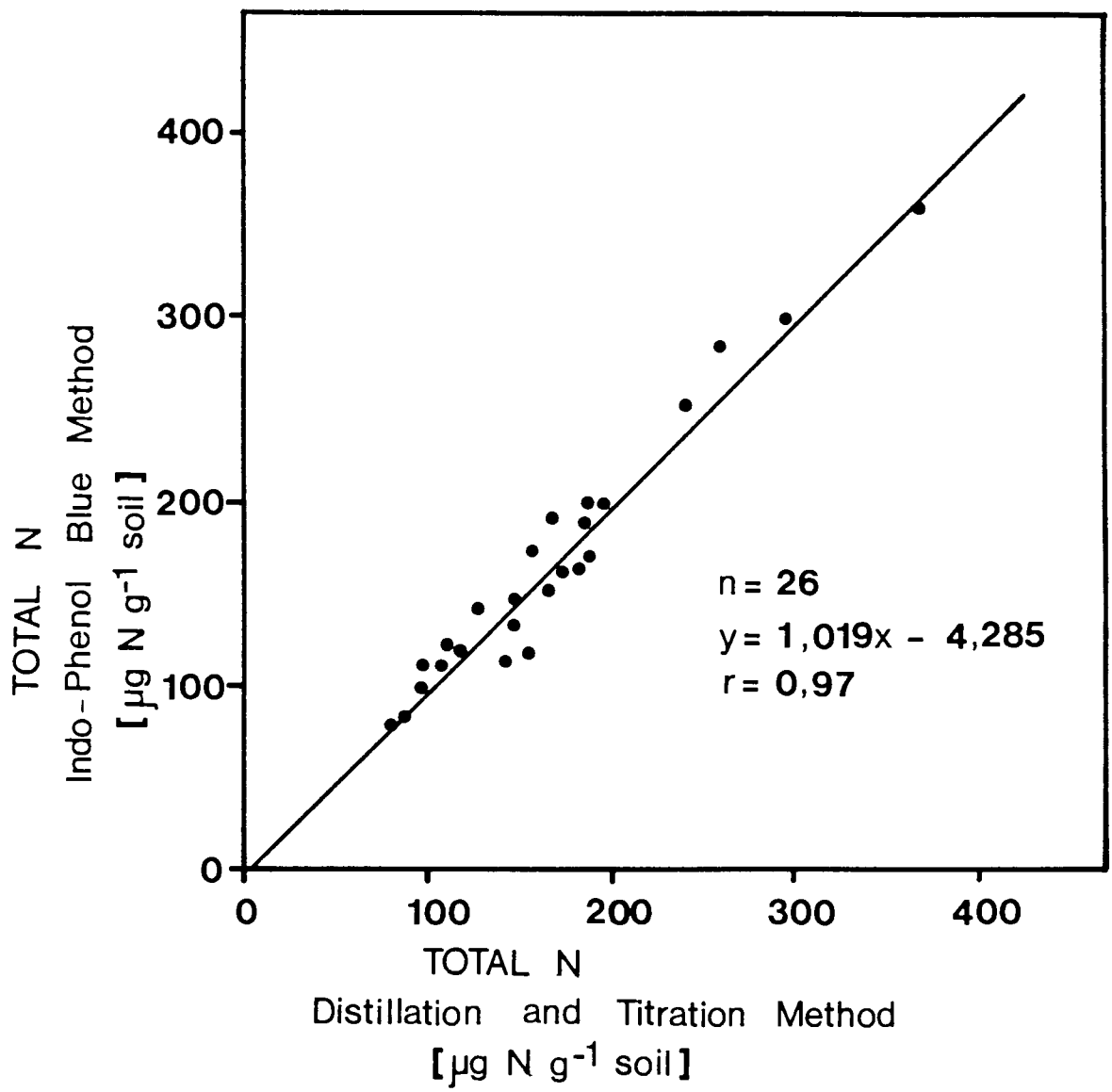


Figure 3.5: The relationship between the indo-phenol blue and distillation and titration methods of ammonium determination in Kjedahl digests.

removal of the moisture content as described in 3.1.7. the crucibles of dry soil were placed in a muffle furnace at 450^{o}C for 16 hours. The samples were cooled in a desiccator and the mass loss determined. Results are expressed as a percentage of the dry mass of the soil.

Total organic carbon was determined by the Soil Science section of the Department of Agriculture, Winter Rainfall Region, using a Walkley-Black technique (Jackson 1958) for oxidizable organic carbon.

3.1.9. Determination of soil pH.

Soil pH determination using electrometric measurement may be undertaken using several extraction procedures involving different concentrations of soil suspension and different salt compositions of the suspending medium (Jackson 1958). Three different extraction methods have been used in this investigation. They are as follows:

a) Soil : distilled H_2O ratio of 1:2,5. To a 10 g sample of soil 25 ml distilled water was added. The suspension was agitated for 30 min after which the pH was determined using a glass electrode with an Orion pH meter.

b) Soil : 0,01M $CaCl_2$ ratio of 1:2,5. To a 10 g sample of soil 25 ml 0,01M $CaCl_2$ was added to mask the

variability in salt content of the soils. After agitation for 30 min (Schofield & Taylor 1955) the pH was determined using a glass electrode with an Orion pH meter.

c) Soil : 1M KCl ratio of 1:2,5. To a 10 g sample, 25 ml 1M KCl was added to overcome changes in pH due to changes in salt content as in the case of the 0,01M $CaCl_2$ samples. After agitation for 30 min the pH was determined as described above.

It should be noted that the pH values obtained were lower in the 0,01M $CaCl_2$ and 1M KCl extraction media than in distilled water because of the influence of the salt composition of the suspending medium (Linder et al. 1984, Peech 1965). In all cases where soil pH is given in the report reference will be made to the solution in which the soil was suspended.

3.1.10. Determination of soil cation content.

Sodium, potassium, magnesium and calcium were determined by the Soil Science section of the Department of Agriculture, Winter Rainfall region, using an ammonium acetate extraction procedure followed by flame emmission or atomic absorption techniques (Hesse 1971).

3.1.11. Soil nitrogen mineralization studies.

Point in time determinations of mineral N in the soil provide little indication of the microbial release or mineralization of N from the organically combined form, in which state practically all soil N occurs. The complexities of the methods used to provide an index of the availability of mineral N have long been appreciated and reviews by Harmsen and van Schreven (1955), Allison (1956) and Bremmer (1965b) have discussed the many biological and chemical methods available for evaluating N mineralization. Stanford and Smith (1972) found that incubation techniques to determine biological soil N mineralization potential provided reliable methods for estimating amounts of N mineralized during selected periods of time under specified temperature and water regimes.

In this study an incubation technique based on mineral N analyses before and after incubation was used (Hopmans et al. 1980, Lamb 1980). Incubation experiments to determine mineralization were designed to investigate two main features of mineral N release in the soil:

a) The effect of fire on potential N mineralization rates in a coastal fynbos soil using a laboratory incubation technique, and

b) The rate of N mineralization in soils beneath

stages of a coastal fynbos succession using both field and laboratory incubations.

3.1.11.1 Soil collection for mineralization experiments.

Soil used to investigate the direct effects of fire on N mineralization were (i) 0 - 7,5cm in depth collected on the 22nd October 1980 (pre-fire soil) and (ii) samples 0 - 7,5cm in depth collected three days after the wildfire which occurred on the 2nd November 1980 (post-fire soil) (Site A Fig. 2.4). A further 0-1 cm deep soil sample was collected on the day of the fire. These soils were air-dried and stored before the incubation experiments were undertaken.

Soils used to study the N mineralization potential of soils from different seral stages of a coastal fynbos succession were collected during December 1981 beneath 1, 6 and 20 year old Protea repens plants. (Sites D, E and F in Fig. 2.4 respectively) Depth category sampled was 0 - 7,5cm in each case. The soils were used fresh on the day of collection for the mineralisation studies.

3.1.11.2 Incubation Studies.

a) Laboratory incubation in polyethylene sealed bottles.

A 20 g soil sample (dry or moist depending on study) was placed in a 150 ml bottle. Water was added to obtain moisture contents in the range from 0, 25, 50,

75, 100 and 150% of field capacity (field capacity (-0,3 bar) of Clovelly soil form determined by pressure plate technique was found to be 2.4% of the dry mass (Fry and Lambrechts pers comm.*)). The incubation bottles were sealed with a polyethylene film (Gladwrap) to allow the diffusion of 0_2, CO_2 and N_2 but to prevent moisture loss. Cook (1958), quoted in Gasser (1961), reported that a 1mm thick polyethylene sheet is permeable to N_2 (diffusion rate 0,95 ml m^{-2} $24h^{-1}$ at 20^oC), 0_2 (diffusion rate 2,75 ml m^{-2} $24h^{-1}$ at 20^oC) and CO_2 (diffusion rate 8,64 ml m^{-2} $24h^{-1}$ at 20^oC) and that no H_20 transfer occurred. The moisture contents of the incubated samples were, nevertheless, checked each week gravimetrically and, if necessary, water was added to maintain the specified moisture content. Different samples were incubated for 7, 14, 28 and 42 days at 10^o, 25^o, 30^o and 50^oC. Samples were extracted after incubation with 80 ml 1M KCl and the extracts analysed for NH_4-N, NO_3-N and NO_2-N as described in Section 3.1.5.

* Fry, M. & Lambrechts, J.J.N., Department of Soil Science, University of Stellenbosch, Stellenbosch, South Africa.

b) Laboratory incubation in polyethylene bags.

A 200 g (dry or moist sample depending on study) soil sample was placed in a polyethylene bag and moisture added to bring the sample up to field capacity. The polyethylene bag was sealed and placed in a plastic flower pot lined with saturated vermiculite. This in turn was sealed within a large loosely tied plastic bag after all six replicates had been added. The samples were then incubated at 30°C for 42 days to estimate potential net N mineralization rate. After the incubation period, 10 g sub-samples were removed and extracted with 1M KCl and the extract analysed for NH_4-N, NO_3-N and NO_2-N as described in Section 3.1.5. A further set of samples were prepared without the addition of water to investigate the N mineralized in the soil under field moisture conditions. This experiment was undertaken during the dry summer month of December 1981.

c) Field incubation.

Samples for incubation in the field were prepared in polyethylene bags as described above (Section 3.1.11.2.b.). The polyethylene bags containing the soil samples, either in the field moist state or adjusted to field capacity, were buried inside plastic flower pots (to prevent animal damage) containing saturated

vermiculite (which was added to maintain a saturated environment) within the pot to prevent further dessication of the sample. The pots were buried at the depths from which they were removed beneath the canopy of the different ages of P. repens where the samples had been collected. After incubation for 42 days the six replicates from each site and depth were returned to the laboratory, extracted with 1M KCl and the extract analysed for NH_4-N, NO_3-N and NO_2-N. The mineral N accumulated over the 42 day incubation period was calculated by subtracting the initial quantities present at the initiation of the incubation.

3.2 Plant collection and plant nitrogen analytical procedures.

3.2.1 Sampling and harvesting of Thamnochortus punctatus.

Five male Thamnochortus punctatus plants (5-6 years old) were selected for phytomass and nutrient distribution studies at approximately 14 week intervals from July 1980 - January 1982 (Site B Fig. 2.3). Criteria for plant selection were (i) isolation from other members of the family Restionacae, as a precaution against confusing the roots of different species and individuals, and (ii) the lack of disturbance of their

immediate environment. The positions of sampled plants within the 1 ha sample area were marked so that neighbouring plants, which may have been disturbed by sampling, were not selected at later sampling dates. In January 1982 ten 15 month old plants and five 3 year old plants were sampled in neighbouring patches of fynbos at Pella which had been burnt during wildfires occurring during November 1980 and March 1979 respectively.

The plants were harvested by severing the above ground parts of the plant at ground level. The material was divided into the following categories on the basis of apparent function and/or age:

1) Developing culms: Young culms which bore no inflorescences or developing inflorescences up to the anthesis stage.
2) Developing inflorescences: Inflorescences prior to and during anthesis.
3) Mature culms: Culms which had borne inflorescences in the most recent flowering season.
4) Matured inflorescences: Post-anthesis inflorescences.
5) Senescing culms: Culms which appeared chlorotic.

6) Dead culms: Completely senesced culms which were brown in colour.

7) Vegetative branches: Finely divided stem and leaf material borne on the culm or on the rhizome itself. Two age groups were recognized, namely mature and dead vegetative branches.

The samples were dried in a forced draught oven at 80^{o}C for 48 hours before dry mass was determined.

The below ground plant parts were sampled by means of a rectangular soil monolith (28cm x 38cm x 10cm) which was centred on the tussock base. The soil monolith containing the root material was then washed through a 2mm sieve. The remaining rhizome and root material was washed further to remove adhering soil particles and sorted by flotation in water into the following categories:

1) Rhizome: Dark brown rhizome and culm occurring just below the soil surface.

2) Developing roots: Young roots with a fleshy cortical layer and a white growing tip. In section a white coloured stele and cortical layer were apparent.

3) Mature roots: Roots from which the cortex had been sloughed off exposing a brown endodermal layer. In cross section a distinct difference between the white stele and light brown endodermis was apparent.

4) Dead roots: Roots dark brown to black in colour and very brittle. No colour difference between stele and endodermis was apparent.

3.2.2 Preparation of plant samples for total N determination.

Methods used in the preparation of plant material for nutrient analysis are designed to maintain (or assumed to maintain), the various chemical constituents unchanged. Drying and other preparative treatments, however, often change the chemical composition of the plant material and hence a standard procedure for the preparation of plant material for nutrient analysis is essential to obtain reproducible results.

Lockman (1980) reports that drying at low (40^{o}C) or high (70^{o}C+) temperatures resulted in greater dry mass loss than intermediate temperatures (50 - 60^{o}C). This he attributed to enzymatic degradation of the plant material at low temperatures while high temperatures

result in thermal decomposition. Haslemore et al. (1980) reported little difference in dry mass of samples dried at 95°C, vacuum dried at 40°C and freeze dried, while recoveries of total nitrogen were highest after heat drying at 95°C for 24 hours and lowest after freeze drying. The decrease in total N in the freeze dried sample has been attributed to loss of proteinaceous dust during the process of comminution (Haslemore et al. 1980).

It was decided in this study to standardise the drying conditions at 80°C for 48 hours, and samples were placed in the forced draught ovens within 24 hours of field sampling. The dried plant material was then ground in a Wiley mill to pass through a 40 mesh screen. Any material remaining in the grinding chamber was removed and mixed with the finely ground material. This was done to ensure that no segregation of more resistant material occurred and that the nutrient analyses of samples reflected the nutrient content of all material. After grinding, the samples were redried at 80°C for 24 hours before being stored in a desiccator.

3.2.3 Total nitrogen in plant material.

3.2.3.1 Micro-Kjeldahl digestion of plant material.

A 0,1000 g oven dried (80°C) plant sample milled to pass through a 40 mesh screen was placed in a 75 ml thick wall digestion tube. The sample was washed to the bottom of the tube with 1 ml of double-deionised water and after standing for 30 min, 3 ml concentrated nitrogen-free AR sulphuric acid (specified gravity 1,84) containing 34 g l^{-1} salicylic acid was added together with 0,2 g sodium thiosulphate and a selenium catalyst tablet (B.D.H, Poole U.K.). Blank determinations were made on the above reagents without the addition of plant material. The digestion was carried out as described in Section 3.1.6.1, after which the digest was cooled and made up to a final volume of 50 ml. Determination of NH_4-N in the digest was undertaken by either a distillation-titration technique (Section 3.1.6.2) or by a colorimetric indo-phenol blue procedure (Allen et al. 1974). Analysis of plant material was performed in triplicate.

3.2.3.2 Colorimetric determination of ammonium in Kjeldahl digests of plant material.

In the past digestion of plant material was very time consuming, but since the introduction of multisample block digestors the most time consuming step

in total N analysis has been that of determining the NH_4-N present in the digest. Techniques available for NH_4-N determination in Kjeldahl digests include the slow distillation and titration method (Section 3.1.6.2) as well as the quicker methods using an ammonia electrode (Gallaher et al. 1976, Mills 1980, Powers et al. 1981) or colorimetry (Eastin 1978, Kubin & Shra 1980, Smith 1980, Nkonge & Ballance 1982). The colorimetric method most commonly adopted for NH_4-N analysis in Kjeldahl digests is the indo-phenol blue procedure because of its sensitivity, simplicity of equipment and the potential to automate the procedure at a later date (Schuman 1973, Eastin 1978, Nkonge & Ballance 1982).

The method adopted in this study was based on that described by Allen et al. (1974) except that a partial neutralisation step was included before colour development was allowed to proceed (Mitchell 1972). The method was as follows: To a 0,5 ml aliquot of Kjeldahl digest sample 2 ml of 0,25M NaOH was added to partially neutralise the samples: a pH of 3 to 4 was recorded after partial neutralisation whereas in the untreated digest a pH < 1 was found. The sample was mixed and allowed to stand for 1 hour. Thereafter the following reagents were sequentially added with a Gilson automatic pipette:

a) 3,2 ml sodium potassium tartrate (10% (w/v)),

b) 0,4 ml sodium nitroprusside (0,16% (w/v)),

c) 0,8 ml sodium phenate reagent prepared fresh each day by dissolving 50 g phenol in 250 ml 40% (w/v) NaOH and diluting to 400 ml,

d) 0,4 ml sodium hypochlorite (with 5% available Cl^-), and

e) 12,7 ml double-deionised water to make the volume up to 20 ml.

The samples and reagents were mixed and after 20 min in a waterbath at 40°C the solutions were cooled and the absorbance determined at 625nm within 10 minutes. Batches of 15 samples, 3 blanks and 5 standards were run simultaneously.

3.2.4 Extraction of nitrate reductase from members of the South African Proteaceae.

W.D. Stock and O.A.M. Lewis

Botany Department, University of Cape Town, Rondebosch, 7700, South Africa.

The enzyme nitrate reductase has been used as an ecological indicator of the ability of plant species to assimilate NO_3-N from the environment (Lee & Stewart 1978). Investigations of nitrate reductase activity in members of the Proteaceae were hampered by the lack of a suitable method to demonstrate the activity of this enzyme due to inhibition by endogenous proteases or polyphenolic compounds. This section reports upon a method which was found suitable to monitor nitrate reductase activity in the Proteaceae and comments upon the enzyme activities in relation to the nutrient environment in which these plants are found. This section was published as a paper in the South African Journal of Botany 1 (4): 124-126 (1982).

INTRODUCTION

Although it has been shown in ^{15}N studies by Lewis and Stock (1978) that nitrate can be used as a nitrogen source in the nutrition of the shoots of Proteaceous plants, it has not been possible to demonstrate the presence of nitrate reductase (NADH dependent) in the leaves and roots of the plants using *in vitro* techniques and conventional extracting media. The inability to obtain an active extract of nitrate reductase (NR) could be due to a number of factors, e.g. the presence in the plant of phytic acids, tannin or hydrolytic and oxidative enzymes (Loomis & Battaile 1966) or simply low levels of nitrate reductase activity, (NRA). Problems in extracting active NR from leaves of members of the Ericaceae (*Vaccinium angustifolium* and *V. macrocarpon*) has resulted in proposals that the enzyme is entirely absent from these plants (Townsend & Blatt 1966, Greidanaus *et al*. 1972). It has subsequently been found in other members of the Ericaceae (*Leucothoe catesbaei* and *Rhododendron catawbiense*) that inhibition of NR activity in extracts was due to the presence in the plant of a galloyl ester-like compound similar in nature to tannic acid (Dirr & Barker 1973). Other plants such as barley and maize are thought to contain a proteolytic enzyme capable of inactivating the enzyme complex by acting on the NADH (NO_3) c R component (Wallace 1974,

Lewis et al. 1982a).

The present study investigates the protective effects of agents added against polyphenolic inhibition and proteolytic enzyme inactivation on in vitro NRA of extracts of shoots and roots of Protea repens and Protea cynaroides. Polyphenolic inhibitors of enzyme extracts have successfully been absorbed by insoluble polyvinylpyrrolidone (POLYCLAR AT,BDH) (PVP) (Loomis & Battaile 1966) while other (Schrader et al. 1974, Sherrard & Dalling 1978, Lewis et al. 1982a) have shown that casein in the extraction medium prevents proteolytic enzyme degredation of active nitrate reductase.

MATERIALS AND METHODS

Plant Material

Eighteen month old plants of Protea repens (L.) L. and Protea cynaroides (L.) L. which had been cultivated in pots in a sand peat mixture with no supplementary nitrogen fertilization were used in the experimentation. Twenty-four hours prior to nitrate reductase extraction the plants were fed 200 ml Long Ashton nutrient solution containing 2mM NO_3-N (Hewitt 1966). Barley (Hordeum vulgare L. cv. Clipper) leaves were obtained from plants germinated and grown in a nutrient film technique trough

using a Long Ashton nutrient solution containing 2mM NO_3-N. Twenty day old leaves were used for all barley assays.

Nitrate Reductase Extraction

Nitrate reductase was extracted from the leaves and roots of fresh Protea spp. by grinding 1 g plant material in a chilled mortar and pestle at 4°C with 12 ml of one of the following extracting media:

a) 0,1M phosphate buffer pH 7,5; 1mM EDTA and 1mM dithiothreitol to which was added 1,5 g of insoluble polyvinylpyrrolidone (BDH, POLYCLAR AT) (Loomis & Battaile 1966).

b) 2,5% soluble casein (BDH), 0,1M phosphate buffer pH 7,5; 1mM EDTA and 1mM dithiothreitol (Lewis et al. 1982a).

The extract was squeezed through a double layer of cheese cloth and the filtrate was centrifuged at 2000 g for 5 min at 3°C. All extractions of barley material followed the method of Lewis et al. (1982a). The efficacy of each NRA protectant was determined by measuring its ability to prevent the loss of nitrate reductase activity in barley leaf extracts when extracts of Protea leaf and root were added to them.

Nitrate Reductase Assay

The reaction mixture for the determination of NRA was as follows: 0,1 ml 1M phosphate buffer pH 7,5;0,1 ml NADH (1 mg ml^{-1}); 0,2 ml 0,1M KNO_3 and 0,2 ml barley extract or 0,3 ml Protea extract made up to a final volume of 2 ml with distilled water.

In assays where the inhibition of barley NRA by the Protea extract was investigated, 0,2 ml barley extract and 0,3 ml Protea extract were added to the reaction mixture before it was made up to final volume. The samples were incubated at 27°C for 15 min and the reaction terminated with 1 ml of 1% (w/v) sulphanilamide in 1,5M HCl and 1 ml of 0,01% (w/v) N-(1-napthyl) ethylenediamine hydrochloride solution. Optical density was read at 540nm after 5 min. Samples containing casein required centrifugation at 2000 g for 5 min to remove the coagulated protein. Triplicate aliquots of extract were assayed in each experiment.

RESULTS AND DISCUSSION

Casein as a NRA protecting agent for Protea extracts.

It is evident from the results shown in Table 3.2 the casein does not protect NRA in extracts of Protea repens leaf and root material. No NRA could be detected in the P. repens extracts and, in addition, the casein

protected extracts of P. repens inhibited nitrate reductase activity in casein protected barley leaf extracts. The leaf extracts of P. repens caused a greater inhibition of barley NRA than did the root extract of the same plant.

These results indicate that the inhibitor responsible for NRA inactivation in Protea extracts is not a proteolytic enzyme as is probably the case in barley, but some other factor that is distributed in greater quantities in the leaf than in the root.

PVP and a NRA protecting agent for Protea extracts

The activity of nitrate reductase extracted from P. repens leaves (protected by PVP), barley leaves (protected by casein) and a mixture of the two is shown in Table 3.3.

From these results it is apparent that when PVP is utilised as a protectant, significant activities of NRA in Protea leaves are obtained. In experiments where Protea extract was added to barley leaf extract no inhibition of barley NRA was detected (barley leaf/protea leaf mixture gave 96% the activity of the sum of the two extracts assayed individually). These results demonstrate the effectiveness of PVP as a protection agent for the extraction of NR from Protea spp. and indicate that the leaves of Protea spp. have a

Table 3.2: Inhibition of barley leaf nitrate reductase activity by extracts of Protea repens leaf and root tissue in the presence of casein. Mean ± SEM.

Plant Extract	Barley Leaf	Barley Leaf + Protea Root	Protea Leaf + Protea Leaf	Protea Leaf	Protea Root
Protectant	Casein	Casein	Casein	Casein	Casein
NR Activity (μmol NO_2 gfw^{-1} h^{-1})	6,9 ±0,23	4,8 ±0,19	2,4 ±0,08	0	0
Percentage Inhibition of Barley Extract	0	29,6	64,6	-	-

Table 3.3: Nitrate reductase activity of *Protea repens* extracts protected with PVP. Mean ± SEM.

Plant Extract	Barley Leaf	*P. repens* Root	*P. repens* Leaf	Barley Leaf + *P.repens* Leaf
Protectant of Protea extract	*	PVP	PVP	* PVP
NR Activity (μmol NO_2 gfw^{-1} h^{-1}	7,7 ±0,14	0,2 ±0,01	1,3 ±0,06	8,7 ±0,30

* Barley always extracted with casein.

Table 3.4: Nitrate reductase activity of Protea repens and Protea cynaroides leaf material. Mean ± SEM.

Plant Extract	Protea repens Leaf	Protea cynaroides Leaf
Protectant	PVP	PVP
NR Activity (μmol NO_2 gfw $^{-1}$ h $^{-1}$)	2,2 ± 0,05	3,7 ± 0,01

low (less than 15% barley leaf NRA) but detectable nitrate reductase activity. The activity of Protea repens root NRA (PVP protected) is also shown in Table 3.3; this is ± 10% the activity of the leaves of this plant.

The in vitro NRA of the leaves of a second species of Protea, Protea cynaroides, was investigated using PVP protection in the extract preparation. The results are shown in Table 3.4 and indicate that the shoots of this plant have very similar NRA to Protea repens.

The results of these experiments indicate that the inhibitor responsible for NR inactivation in Protea spp. is probably not a proteolytic enzyme as in the case of barley, but a polyphenolic constituent of the plant that is distributed in greater quantities in the leaf than in the root. It is well known that Protea spp. have a high content of polyphenolic compounds as they were once used widely in the Cape Province, South Africa, as domestic tanning agents (Williams 1930, Wehmer 1931). Shoot NRA in the Proteaceae is low (2-4 μmol NO_2 h^{-1} gfw^{-1}) when compared with nitrophilous plants such as Zea mays (9,3 μmol NO_2 h^{-1} gfw^{-1}, Sherrard & Dalling 1978), Hordeum vulgare (14,8 μmol NO_2 h^{-1} gfw^{-1}, Lewis et al. 1982) and Helianthus annuus (24,7 μmol NO_2 h^{-1} gfw^{-1}, Kaiser & Lewis 1981) and is possibly an ecophysiological characteristic of those members of the

Proteaceae which are restricted to the low nutrient soils of the South Western Cape, South Africa.

ACKNOWLEDGEMENTS

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3.2.5 Design of ^{15}N feeding experiments to determine plant N uptake and assimilation.

3.2.5.1 Plant material.

Seeds of Protea repens (L) L. were collected at the Fynbos Biome Research Site at Pella after a fire that occurred on the 2nd November 1980. The seeds were sown in a Clovelly soil (obtained from Pella) and on the 14th day after germination the seedlings were transplanted into pots containing acid washed sand. The Protea repens seedlings were grown for 3 months in a well ventilated glasshouse and watered with tap water every 2 or 3 days until the cotyledons were chlorotic, indicating that all internal nutrient reserves had been utilized. The plants were then watered every 3 days with 50 ml of 10-fold diluted standard Long Ashton solution containing a mixture of 0,1 mM NO_3-N and 0,1 mM NH_4-N (Hewitt 1966). After 14 days on this nutrient regime 9 plants were fed every 2 days with 50 ml of 10-fold diluted standard Long Ashton solution containing 2mM $^{15}NO_3$-N at pH 5 and a further 9 plants with the diluted standard Long Ashton solution at pH 5 containing 2mM $^{15}NH_4$-N. The pot leachate from the $^{15}NH_4$-N fed plants was analysed to ensure that nitrification was not occurring, thereby altering the N form being fed to the plant. After 3, 6 and 9 days 3 plants from each feeding regime were

sampled and soluble and bound N compounds extracted for determination of ^{15}N enrichment.

3.2.5.2 Preparation of samples for ^{15}N determination.

The preparation of samples for ^{15}N analysis followed different pathways for NO_3-N and NH_4-N feeding regimes as shown in Figures 3.6 and 3.7.

a) Extraction of nitrogenous compounds.

The extraction and filtration procedure was common to both feeding regimes. The fresh mass of the root and shoot plant parts of the seedling were determined immediately on harvesting. The material was finely shredded and frozen with liquid N_2. The frozen brittle plant material was ground in a mortar and pestle and after the N_2 had evaporated, 50 ml of 80% (w/v) cold ethanol g^{-1} fresh mass was added. A final homogenisation was performed with an Ultra-Turrox homogenizer. The sample flasks were sealed with Parafilm wax sheet and placed in a cold room at $0^{o}C$ for 24 hours to allow the extraction of soluble N compounds. The homogenate was filtered through Whatman No 1 filter paper. The solid residue and filter paper were carefully washed with 80% ethanol. The filtrate was then evaporated under an air stream to obtain a final volume of 10 ml. A 5 ml aliquot of petroleum ether was shaken with the sample to extract lipid and chlorophyll material. The sample was frozen at

-20°C and the petroleum ether decanted. This sample contained the soluble N compounds. The solid material remaining on the filter paper was dried at 80°C (bound or insoluble N fraction).

b) Preparation of bound N fraction for ^{15}N analysis.

The solid residue of the homogenised plant sample was collected on the filter paper, placed in an oven at 80°C for 24 hours. Thereafter the dry matter was carefully removed from the filter paper and its dry mass determined. A 0,1000 g dry mass sample was added to a Kjeldhal digestion tube and the digestion and distillation process described in Section 3.1.6.2 was followed. The NH_4-N from the distillation was collected in 0,02M HCl and the quantity of N present was calculated by titration against 0,005M NaOH. The sample was re-acidified with 0,5 ml of 0,1M HCl and evaporated down, on a warm hotplate, under an airstream so that a final volume of 0,2 ml for every 20 µg N was obtained. The samples were stored frozen until ^{15}N analysis was undertaken.

c) Separation of N compounds in the soluble fraction.

It was necessary to remove the mineral N sources that the plant had absorbed but not yet metabolised before the rate of N assimilation by the plant could be calculated. The flow diagrams Figures 3.6 and 3.7 show

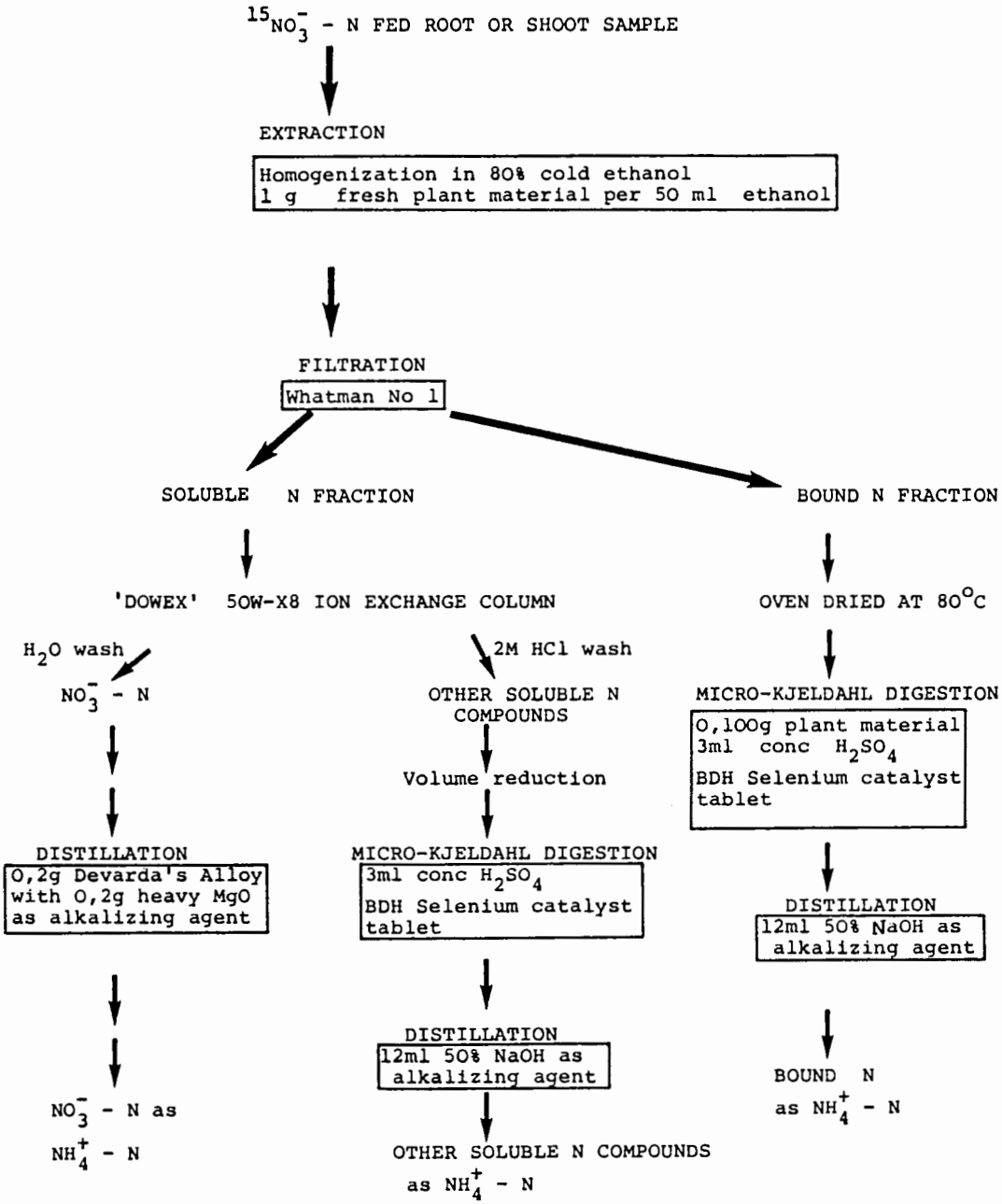


Figure 3.6: Flow chart of the techniques used to prepare $^{15}NO_3$-N fed plant samples for ^{15}N atomic emission determination.

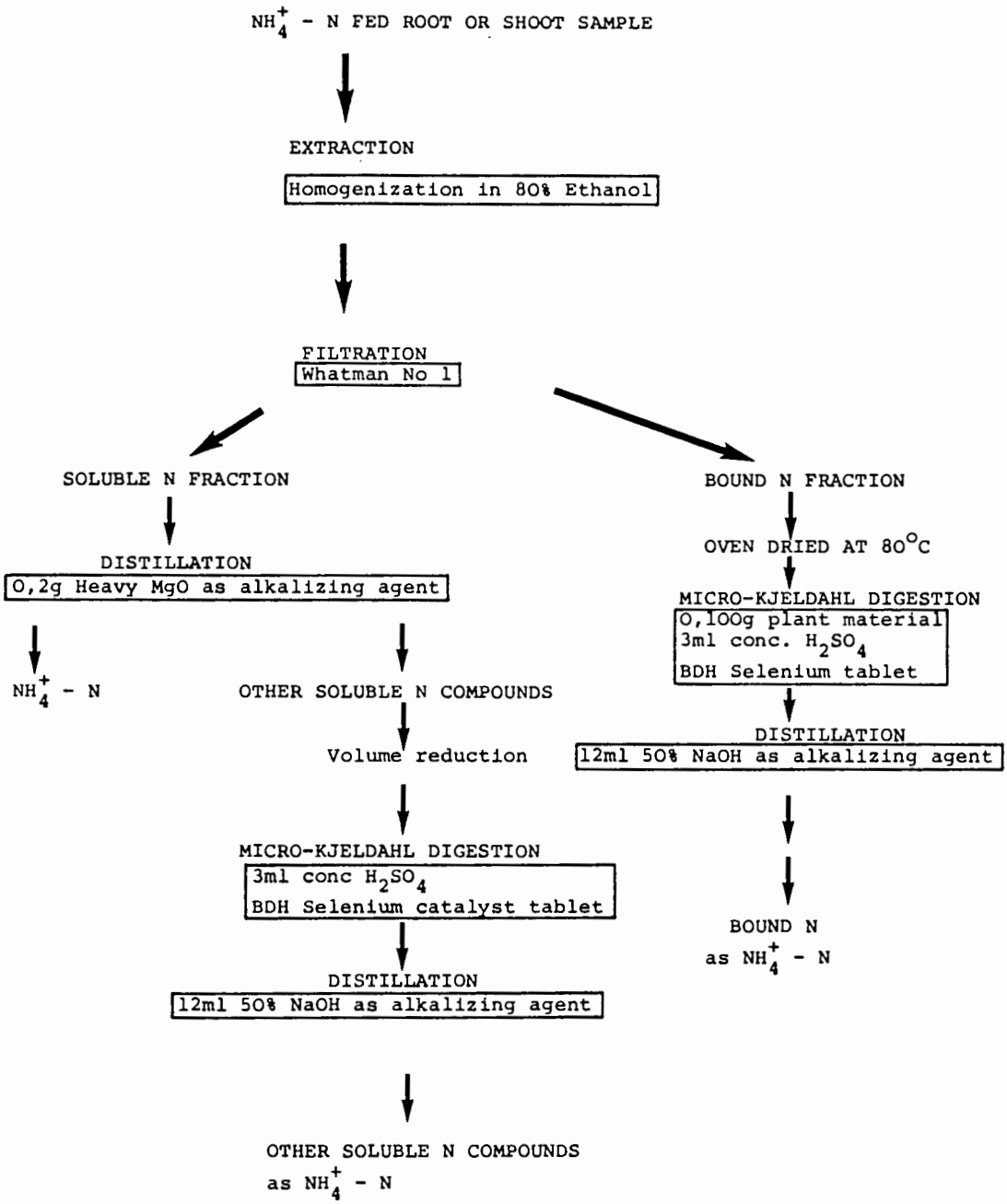


Figure 3.7: Flow chart of the techniques used to prepare $^{15}NH_4$-N fed plant samples for ^{15}N atomic emission determination.

the different techniques used to separate the mineral N from the other soluble N compounds.

i) Nitrate fed plants.

Separation of Nitrate.

Separation of nitrate from the soluble N pool was accomplished using an ion exchange resin column as described by Atkins and Canvin (1971). About 50 g of 'Dowex' 50-X8 standard H^+ 100-200 mesh ion exchange resin was treated to remove soluble material, which might interfere with later analysis, by suspending it in 100 ml of 2M HCl and heating to 100°C with continuous stirring. The resin was allowed to cool and the supernatant discarded. This process was continued until the supernatant was clear. The resin slurry was then poured into 1 cm diameter columns plugged at the bottom with glass wool until a resin height of 3 cm was obtained. The column was treated with 20 ml of 2M HCl and then washed to neutrality with distilled H_2O.

The soluble N sample (10 ml) was loaded onto the column and the NO_3-N fraction collected by allowing 50 ml of distilled H_2O to flow through the resin bed, washing out the nitrate which was collected in a 50 ml flask. The soluble N compounds remaining on the column were eluted by slowly pouring 100 ml 2M HCl through the ion exchange resin and collecting the eluate in a 100 ml

beaker. The N collected in each fraction was then converted to ammonia prior to ^{15}N analysis as described below.

Reduction of nitrate to ammonium.

Nitrate was reduced to ammonia by Devarda's alloy, the ammonia being subsequently distilled into standard acid and determined by titration (Fig. 3.6).

The nitrate sample collected from the ion exchange resin was decanted into the flask of the steam distillation apparatus. The flask containing the nitrate sample was then attached to the distillation apparatus and 0,2 g MgO ("heavy powder" ignited at 600^{o}C to remove CO_2 from $MgCO_3$ impurities present, and then stored in a desiccator with silica gel and KOH before use) and 0,2 g Devarda's alloy were added. The distillation was commenced and 30 ml of distillate collected in 0,02M HCl. The distillate was titrated against 0,005M NaOH using Tashiro's indicator and the N content determined (1 ml 0,005M NaOH = 70 µg N). The sample was slightly reacidified with 0,5 ml of 0,1M HCl (to prevent loss of NH_3) and blown down with mild heat under an airstream so that a final volume of 0,2 ml for every 20 µg N was obtained. The samples were stored frozen until ^{15}N analysis was performed.

Conversion of soluble organonitrogen compounds to ammonia.

The soluble N compounds eluted from the ion-exchange column with 100 ml 2M HCl were converted to ammonia using the Kjeldahl digestion procedure. The sample was evaporated in a fume cupboard on a hot plate to a volume of approximately 10 ml. This was transferred to a Kjeldahl digestion tube where 3 ml of AR H_2SO_4 (concentrated, N free) and a mercury catalyst tablet (B.D.H. Poole, U.K.) were added. After the remaining water had been driven off the standard digestion procedure was followed (Section 3.1.6.1). Upon completion of digestion the sample was cooled and ammonia distilled over into a standard acid by alkalising the sample with 12 ml 50% (w/v) NaOH containing 2,5% (w/v) sodium thiosulphate (Section 3.1.6.2) to prevent loss of ammonia through its binding with the mercuric oxide precipitated on the addition of alkali (McKenzie & Wallace 1954). The sample was re-acidified with 0,5 ml 0,1M HCl and blown down with mild heat under an airstream so that a final volume of 0,2 ml for every 20 µg N was obtained. The samples were stored frozen until ^{15}N analysis was performed.

ii) Ammonium fed plants.

Separation of ammonium.

Bremner (1965c) recommends a procedure for the separation of free ammonium from the ammonium of other soluble organo-N compounds in a sample. The recommendation is based on the finding that free ammonium in solutions containing glutamine and other alkali-labile N compounds can be determined quantitatively (i.e. without release of NH_3 from the alkali-labile compounds) by steam distillation of the sample for 2-4 min in the presence of heavy MgO powder.

The procedure adopted in this project was as follows: The 10 ml sample of soluble N compounds obtained upon extraction of the plant material was placed in a distillation flask. This was attached to the distillation apparatus and 0,2 g heavy MgO powder was added. Steam distillation was continued until 30 ml of distillate was collected, which took approximately 4 min. The ammonia was collected in 0,2M HCl titrated against 0,005M NaOH using Tashiro's indicator and the N content determined. After re-acidification with 0,5 ml 0,1M HCl the samples were blown down to a final volume of 0,2 ml for every 20 µg N present. The samples were stored frozen until ^{15}N analysis was undertaken.

Conversion of soluble organonitrogen compounds to ammonia.

The residue from the MgO distillation was evaporated to 10 ml and the soluble compounds converted to ammonia by Kjeldahl digestion as described in Section 3.1.6.1.

3.2.5.3 ^{15}N Determination.

The ammonia present in the samples from the different preparation pathways described was oxidised with sodium hypobromite to release nitrogen gas following the method of Faust (1967). Nitrogen gas was released under vacuum according to the reaction:

$2NH_3 + 3NaOBr \rightarrow N_2 + 3H_2O + 3NaBr$ (Kamen 1957).

The vacuum apparatus for preparation of discharge tubes of N_2 gas consisted of a Edwards high vacuum pump to establish a pre-vacuum (0,1 Pa) connected with a mercury diffusion pump, to bring the system to a final pressure of 1 Pa. Water vapour pressure within the system was reduced by two liquid nitrogen cold traps.

The discharge tube containing the sample as N_2 was excited in a Statron (Packard) NOI-4 atomic emission spectrophotometer (A.E.S.) and a chart recording of the bandheads emitted by the isotopic molecules at 316,5nm for $^{15}N\ ^{15}N$, 316,2nm for $^{14}N\ ^{15}N$ and 315,9nm $^{14}N\ ^{14}N$ was made. A typical trace is shown in Figure 3.8.

A normal excitation produced a red/violet colour

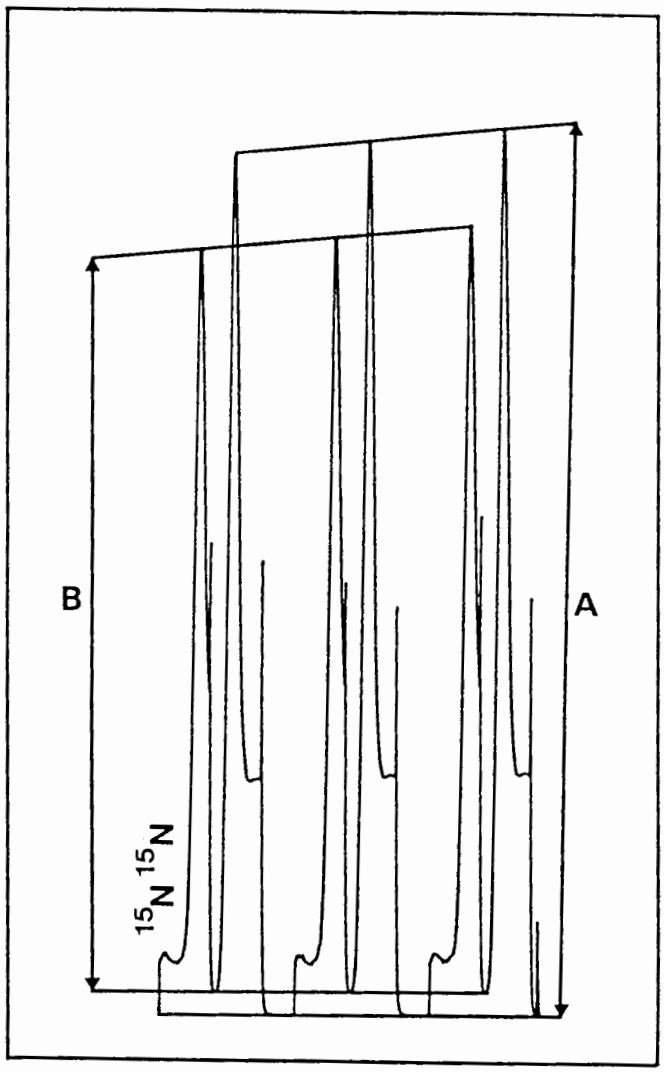


Figure 3.8: A typical trace from the Statron emission spectrophotometer showing good separation of the three dinitrogen species, $^{15}N^{15}N$, $^{14}N^{15}N$ and $^{14}N^{14}N$. The peak heights A and B represent the emissions from the $^{14}N^{14}N$ and $^{14}N^{15}N$ bandheads respectively. The peak height corresponding to the $^{15}N^{15}N$ molecule is very small due to low ^{15}N enrichment of this sample.

whereas white and blue colours indicated contamination by either water vapour or bromine. In these cases, if enough sample remained, new discharge tubes of N_2 were prepared to obtain the correct coloured discharge. The ^{15}N enrichment of the sample was calculated from the formula:

$$\%En = \frac{100}{2(A/B \times Vb/Va) + 1}$$

where A and B were the peak heights of the $^{14}N\ ^{14}N$ and $^{15}N\ ^{14}N$ (Figure 3.8) respectively, and where Va and Vb were the gain settings on the spectrophotometer at which bandheads A and B were recorded. The ^{15}N enrichment was corrected using a standard calibration curve constructed by periodically running a set of calibration standards prepared from $^{15}NH_4Cl$ as shown in Figure 3.9. The percentage enrichment in excess of the natural abundance (A%E) was calculated by subtracting the natural abundance (0,37%) from the corrected percentage enrichment. Enrichment results were expressed as $\mu g\ ^{15}N\ g^{-1}$ fresh mass.

3.3 Atmospheric deposition collection and bulk precipitation nitrogen analytical procedures.

3.3.1 Bulk precipitation collection.

Bulk precipitation (wet fall plus dry fall) samples

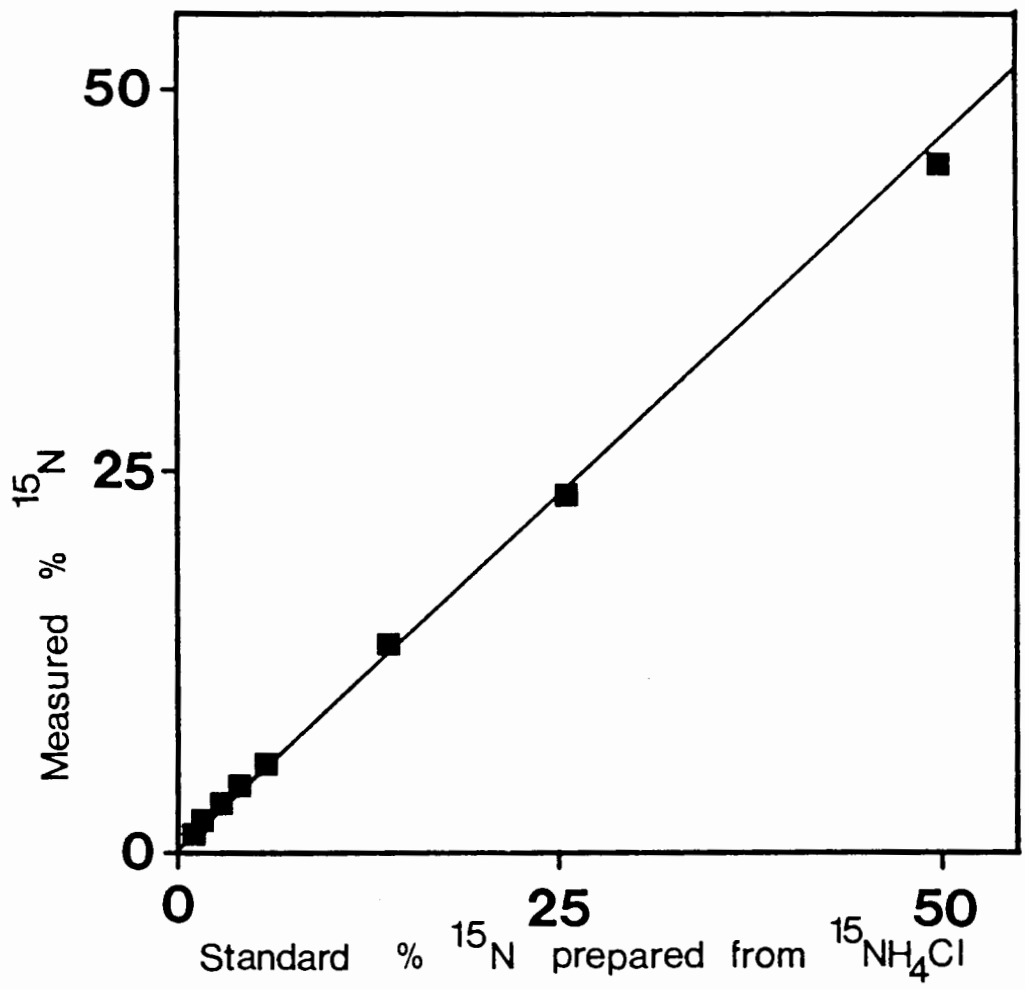


Figure 3.9: Standard curve for the correction of ^{15}N enrichments determined with the Statron atomic emission spectrophotometer.

for nutrient analysis were collected at weekly intervals from the Pella research site for the period June 1980 until June 1982 (Site C in Fig. 2.3). Precipitation was collected in 5 polythene funnels 16,8cm in diameter, each connected to a 2,5 l collecting bottle. The funnels were surrounded with metal spikes to prevent birds from perching and were covered with a $1mm^2$ fibre glass mesh to prevent contamination from insects and plant debris. The funnel lip was 1,5m above soil level and plant canopy height in the study area was 1m.

The funnels and bottles were acid washed followed by a chloroform wash and two rinses of double deionised water before being placed in the field. To each collecting vessel 0,5 ml of a preservative solution was added (500 $\mu g\ ml^{-1}$ mercuric chloride prepared in 1M HCl). During rainfall periods the collecting vessels were sampled at weekly intervals, 50 ml aliquots being removed for analysis. If no rainfall occurred, 30 ml double deionised water was added to rinse the funnel and collecting bottle. This solution was then analysed for the soluble and total input from dryfall.

The funnel and collecting vessel were washed after each sample collection in the manner described above and returned to the field. Any samples showing obvious signs of contamination by bird excrement or insects were discarded and the collecting equipment cleaned and

replaced in the field. From June 1981 a further five collecting vessels were placed in the field 4m above ground level. Sampling of these vessels occurred at weekly intervals in the same manner as described for the collection at 1,5m above ground level.

All samples collected were returned to the laboratory and stored at $4^{o}C$ for 4 to 6 weeks until analysis.

3.3.2 Determination of nitrate in bulk precipitation.

Nitrate was determined by Szechrome NAS analytical reagent following the method described in Section 3.1.5. Nitrate standards were prepared in double deionised H_2O in the range from 0,2 µg NO_3-N ml^{-1} to 1,5 µg NO_3-N ml^{-1}.

3.3.3 Determination of ammonium in bulk precipitation.

Ammonium in precipitation was determined by the indo-phenol blue procedure as described in Section 3.1.5. The only alteration to the method made was that a 3 ml precipitation sample volume was used instead of a 2 ml volume. The total volume was still made up to 10 ml so this did not affect the analytical procedure. Standards of NH_4-N were prepared in double deionised water in the range from 0,1 µg NH_4-N ml^{-1} to 1,5 µg NH_4-N ml^{-1}.

3.3.4 Determination of total N in bulk precipitation.

Total N in precipitation was determined every 3 to 4 months (in the first year) on weekly samples bulked together in proportion to the rainfall volume. In weeks where less than 1mm or no rainfall occurred 1ml of collector rinse water was added. The bulked sample was divided into three aliquots and to each aliquot, 3ml concentrated (NH_4-N free) AR H_2SO_4 was added. Approximately 0,5 g of Devarda's alloy was added to each of the replicates to reduce NO_3-N to NH_4-N and the samples were each evaporated down to 5ml. This concentrate was then decanted into a Kjeldahl digestion tube and a selenium catalyst tablet containing K_2SO_4 (BDH, Poole, UK.) was added. After the remaining water had been driven off a Kjeldahl digestion was carried out as described in Section 3.1.6.1. Ammonium in the digest was determined by collection in 0,02M HCl after alkalisation of the digest with 50% (w/v) NaOH.

The distillation procedure for ammonium determination in the digest was used in preference to the indo-phenol blue technique because of the presence of copper in the Devarda's alloy which was used initially to reduce NO_3-N to NH_4-N. Both mercury and copper are known to interfere with colour development in the indo-phenol blue method (Smith 1980, Ngo et al. 1981).

The quantity of each form of N in wet precipitation was calculated from the concentration of that form multiplied by the volume of rainfall recorded in a standard rain gauge located on the site. Dryfall was calculated as the total content of that N form in the 30 ml of rinsing water.

CHAPTER 4

SOIL NITROGEN AND THE ROLE OF FIRE AS A MINERALIZING AGENT IN A SOUTH AFRICAN COASTAL FYNBOS ECOSYSTEM.

W.D. STOCK and O.A.M. LEWIS

Botany Department, University of Cape Town,

Private Bag, Rondebosch, 7700, South Africa.

Research in various ecosystems of the world has shown the soil compartment of the N cycle to be the largest store of biologically active N. The size of this store is often characteristic of specific ecosystems and the dynamics of soil N changes in quantity and form are often used in describing the functioning of an ecosystem. In the chapter the size of the soil N compartment of a coastal fynbos ecosystem and the relative contribution of each N form is reported. Investigations of the seasonal characteristics of soil N within the ecosystem are described. The importance of fire disturbance on coastal fynbos functioning is evaluated in this chapter by considering fire induced changes in soil N form, quantity and seasonality in soils beneath such vegetation. This chapter has been accepted as a paper for publication in the Journal of Ecology.

INTRODUCTION

Coastal and mountain fynbos vegetation of the South African mediterranean-climate zone occurs on acidic sandy soil of low nutrient status. The role of nutrients, in particular nitrogen and phosphorus, as determinants of vegetation structure and function in mediterranean-type ecosystems is an extremely important one; this topic has recently been reviewed by Kruger et al. (1983).

Controlled burning has been adopted as a fynbos management tool to maintain species richness, to improve water yields in catchment areas,to eradicate alien invasive vegetation, to control levels of combustible fuel and to alter the veld for domestic livestock grazing (Bands 1977, van Wilgen & Kruger 1981). It is well known that fire can affect soil N levels directly by volatilization or by oxidation of organic N in the surface layers. Soil N levels can futher be affected by deposition of ash on the soil surface from combusted plant and litter material (St. John & Rundel 1976, Raison 1979, Mroz et al. 1980, Raison 1980). Fire also has an indirect effect on soil N levels by altering the chemical and physical properties of soil which in turn influence transformations of nitrogen (Sharrow & Wright 1977, Raison 1979). An understanding of the changes in N cycling resulting from fire is therefore essential for successful management of fynbos ecosystems.

Conflicting reports have been made concerning the effects of fire on total soil N. Increased total and available soil nitrogen has often been reported following fire (Mayland 1967, Christensen

& Muller 1975) while decreases in total N have been reported by DeBano and Conrad (1978) and Christensen (1973,1977) (see also Ahlgren & Ahlgren 1960 for an earlier review). Studies on available nitrogen after fire have shown mineral NH_4-N and NO_3-N to be present in greater quantities in burned soils than in soils of comparable unburned sites (Christensen 1973, Christensen & Muller 1975, Sharrow & Wright 1977) although the amount and duration of the increase is highly variable. This lack of unanimity between experimental results has been attributed to the non-uniformity of fires. The amount of N volatilized of deposited as ash results from various fire characteristics caused by differences in mass and spatial distribution of vegetation, the degree of combustion and the subsequent transport by wind or water of the burned residues (Raison 1979).

The investigations reported in this paper have been designed to investigate the following aspects of the soil component of nitrogen cycling in the fynbos with particular reference to the points discussed above:

(a) The spatial heterogeneity and seasonal variations of different forms of nitrogen in unburned soils of a mature 20 year old coastal fynbos site at Pella, South Western Cape Province, South Africa.

(b) The immediate and long term changes in N form, distribution and seasonal variation after fire.

STUDY AREA

The study was carried out 62km north of Cape Town at the 269 ha Pella research site located on the Burgherspost Farm (33° 31'S:18°32'E, altitude 160-220m) in the Malmesbury district of the Cape Province, South Africa. The vegetation consists of coastal fynbos dominated by evergreen shrubs and Restionaceae growing on sandy, well drained, acidic, low nutrient soils with a depth of 2m. Following the South African soil classification system, the soil studied was recognized on the basis of the topsoil and subsoil horizon sequences as a Clovelly soil form (MacVicar et al. 1977).

This soil has been shown by Mitchell et al. (1984) to be of low phosphorus status, probably associated with a low total P content. The vegetation in the 2 ha sampling area was 20 years old, dominated by Protea repens L. and P. burchellii Staph.. It was burnt by a moderately intense wildfire in November 1980. Other important species in the mature stand were Phylica cephalantha Sond., Leucospermum parile L., Thamnochortus punctatus Pill. and Staberhoa distachya (Rottb.). The climate following the Köppen classification, is a true Mediterranean climate (Csa & Csb) which is characterised by dry summers and wet winters (Schulze1947). The 40 year mean rainfall for the Burgherspost Farm is 577mm and the mean annual temperature at Philadelphia, a nearby weather station, (33°40'S: 18°35'E) was 17,3°C for the 5 year period from 1975-1980.

METHODS AND MATERIALS

Soil Sampling

Soil samples were collected for the investigation of spatial (vertical), seasonal and fire induced changes in soil N on a monthly basis from October 1979 until November 1981. Sampling sites were chosen within the 2 ha site by locating undisturbed areas forming open spaces between individual plants of two or more of the following species: Phylica cephalantha, S. distachya, L. parile, T. punctatus of Protea repens. Pits of surface area 1m x 1m and 1,2m in depth were dug every 2 months and soil samples removed from the four faces of the pit in the following depth categories: 0 to 2,5 cm, 2,5 to 7,5 cm, 7,5 to 12,5 cm, 12,5 to 17,5 cm, 17,5 to 22,5 cm, 27,5 to 32,5 cm, 37,5 to 42,5 cm, 67,5 to 72,5 cm and 97,5 to 102,5 cm. These categories are referred to by their depth midpoints (0, 5, 10, 15, 20, 30, 40, 70, 100) except for the surface 2,5 cm which is called 0. Soil samples collected for the intermediate months were from pits 30 cm deep with five depth categories between 0 and 22,5 cm.

The spatial (lateral) distribution of organic and inorganic N in the soil of the rhizosphere regions of selected fynbos plants was investigated during November 1979. Six cores 6,7 cm in diameter and 10 cm in depth were collected from soil beneath bare patches and from soils under the canopies of each of the following plants: L. parile, S. distachya, P. repens and P. cephalantha.

To obviate possible complications caused by diurnal changes in soil N form and concentration, samples were collected between 09 00 and 11 00 hours. Samples were transported to the laboratory on the day of collection and extracted immediately with 1M KCl as described by Stock (1983) for the determination of inorganic N. Moisture content, organic matter content and total N were also determined as described below. Sample pits were refilled and identified by inserting markers to prevent resampling of the same area.

Soil Analyses

Each sample was sieved through a 2mm mesh and thoroughly mixed. Nitrate, ammonium, nitrite and moisture content were determined on fresh soils whereas total N was determined on air-dried soil.

Nitrate, nitrite and ammonium were extracted with 1M KCl and determined by colorimetric procedures. A copper cadmium reduction of nitrate (Bate & Heelas 1975) followed by nitrite determination using the Griess-Ilosvay method was employed. Negligible concentrations of NO_2-N (0,1 μg N g^{-1} soil) were found in the soils investigated, therefore the results obtained from the Cu/Cd reduction method were not corrected for the presence of NO_2-N. Ammonium was determined by a phenol-hypochlorite (Berthelot) procedure modified from Allen et al. (1974) as described by Stock (1983). Total N was determined by Kjeldahl digestion of 2 g air-dried soil samples moistened with 1 ml of double-deionized water before the addition of 3 ml N-free

concentrated H_2SO_4 containing 34 g l^{-1} salicylic acid. A selenium catalyst tablet (BDH, Poole, U.K.) and 0,2 g sodium thiosulphate were added. The use of salicylic acid and sodium thiosulphate was adopted to include the assay of nitrate and nitrite (Bremner 1965d). After digestion on an aluminium block digestor the sample was made up to 50 ml with double-deionized water and the NH_4-N content was determined by a phenol-hypochlorite method as described by Allen et al. (1974).

Moisture content of the fresh soils was determined as mass loss by an 8 g soil sample dried in a forced draught oven at 105°C for 24 hours. The oven-dry soil was used to determine organic matter content which was calculated as the mass lost upon ignition at 450°C in a muffle furnace over 16 hours. All N values obtained were corrected from fresh mass and air-dried mass of soil to oven-dried mass.

Statistical Analysis

Analysis of variance was computed to compare variations between months and to detect difference occurring as a result of fire. Computation was undertaken using a STATJOB ONEWAY and a BIOMEDICAL COMPUTER PROGRAMS PV1 statistical package (Dixon & Brown 1979) on a Univac 1100 computer at the University of Cape Town. Probability values were obtained from tables of critical values of the F distribution as given by Zar (1974). Soil NO_3-N and NH_4-N values were converted to log transformations as the method of Box and Cox (1964) indicated that this procedure was needed to justify the assumptions required for the analysis of variance.

Three-dimensional graphs of soil nitrogen in relation to month and depth were generated by interpolation of gridded data using a SACLANT graphics package (Diederiks 1979).

RESULTS

Spatial heterogeneity of soil nitrogen

Total N concentrations showed significant ($p < 0,01$) vertical decreases from the surface (286 $\mu g\ N\ g^{-1}$ soil) to the subsoil where values of 95 $\mu g\ N\ g^{-1}$ soil at 200 cm were found (Fig. 4.1). The surface 10 cm (5% of total depth) contained 273 kg N ha^{-1} which was 12% of the soil total N content down to a depth of 200 cm (2291 kg N ha^{-1}). A linear relationship existed between the vertical distribution of total N and organic matter content down the profile to a depth of 100 cm (Fig. 4.1). Nitrate and exchangeable NH_4-N showed similar significant decreases in concentration with depth, from 1,2 $\mu g\ N\ g^{-1}$ soil and 2,6 $\mu g\ N\ g^{-1}$ soil respectively to both less than 0,2 $\mu g\ N\ g^{-1}$ soil at 200 cm (Fig. 4.2). Ammonium content (12,3 kg N ha^{-1}) greatly exceeded NO_3-N content (6,0 kg N ha^{-1}) to a depth of 200 cm when samples were taken during October 1980.

The lateral heterogeneity of soil N in the surface 10 cm of the soil in the rhizosphere regions of selected plants and in bare patches showed no significant variations ($p > 0,05$) in total N concentrations which ranged from 259 to 321 $\mu g\ N\ g^{-1}$ soil (Table 4.1). Nitrate and exchangeable NH_4-N concentrations showed significant ($p < 0,05$) differences between soil samples

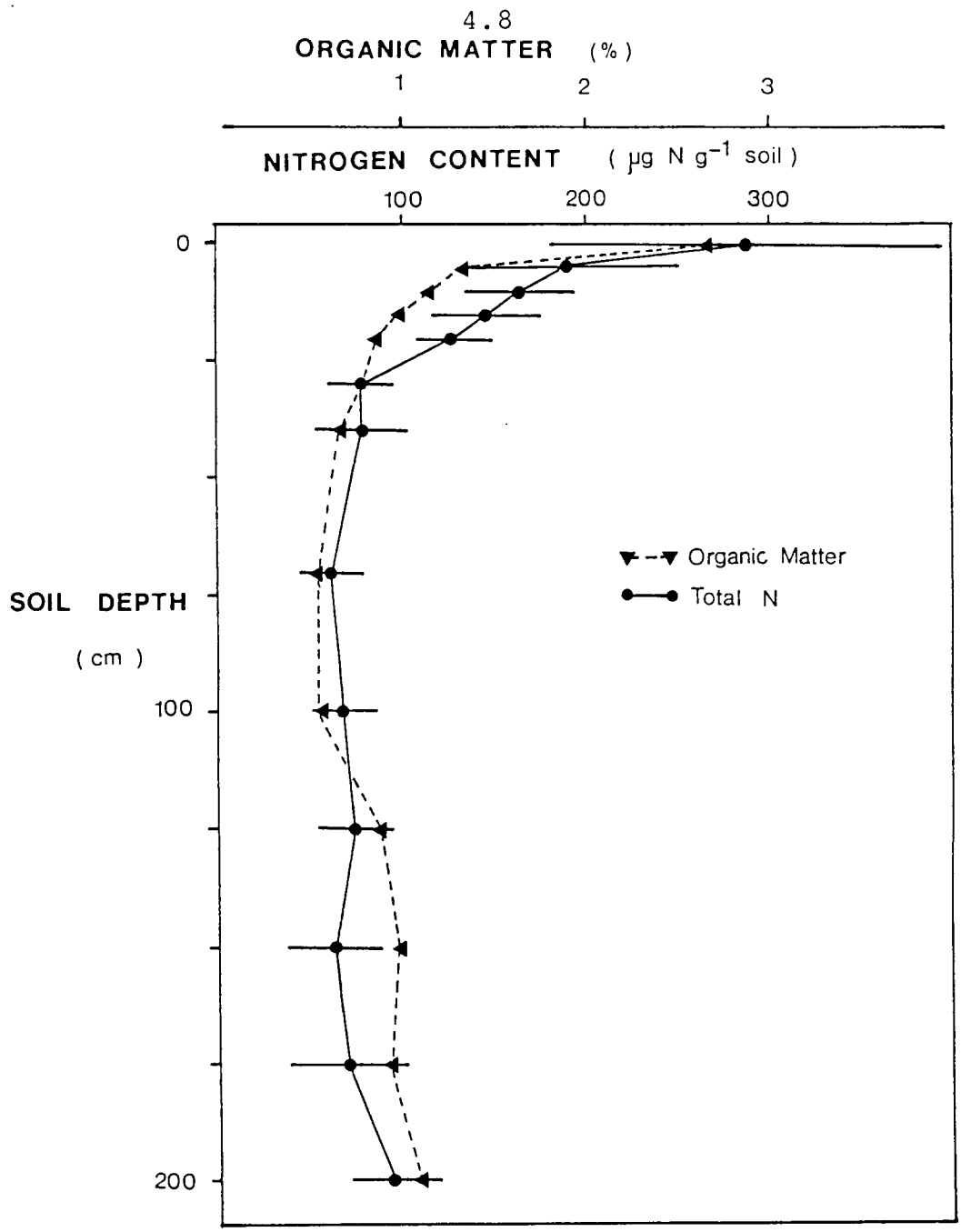


Figure 4.1: The vertical distribution of total nitrogen (mean ± SEM) and organic matter concentrations to a depth of 200cm in a Clovelly soil form beneath coastal fynbos at Pella,South Africa. A linear relationship existed between total N and organic matter concentrations in the surface 100cm. The relationship was Total N = -126,9 + 44,9 (arcsin $\sqrt{\text{organic matter}}$), r^2= 0,97. One way ANOVA showed that total N concentrations were significantly different with soil depth (F = 11,53; df = 12,66; p 0,01).

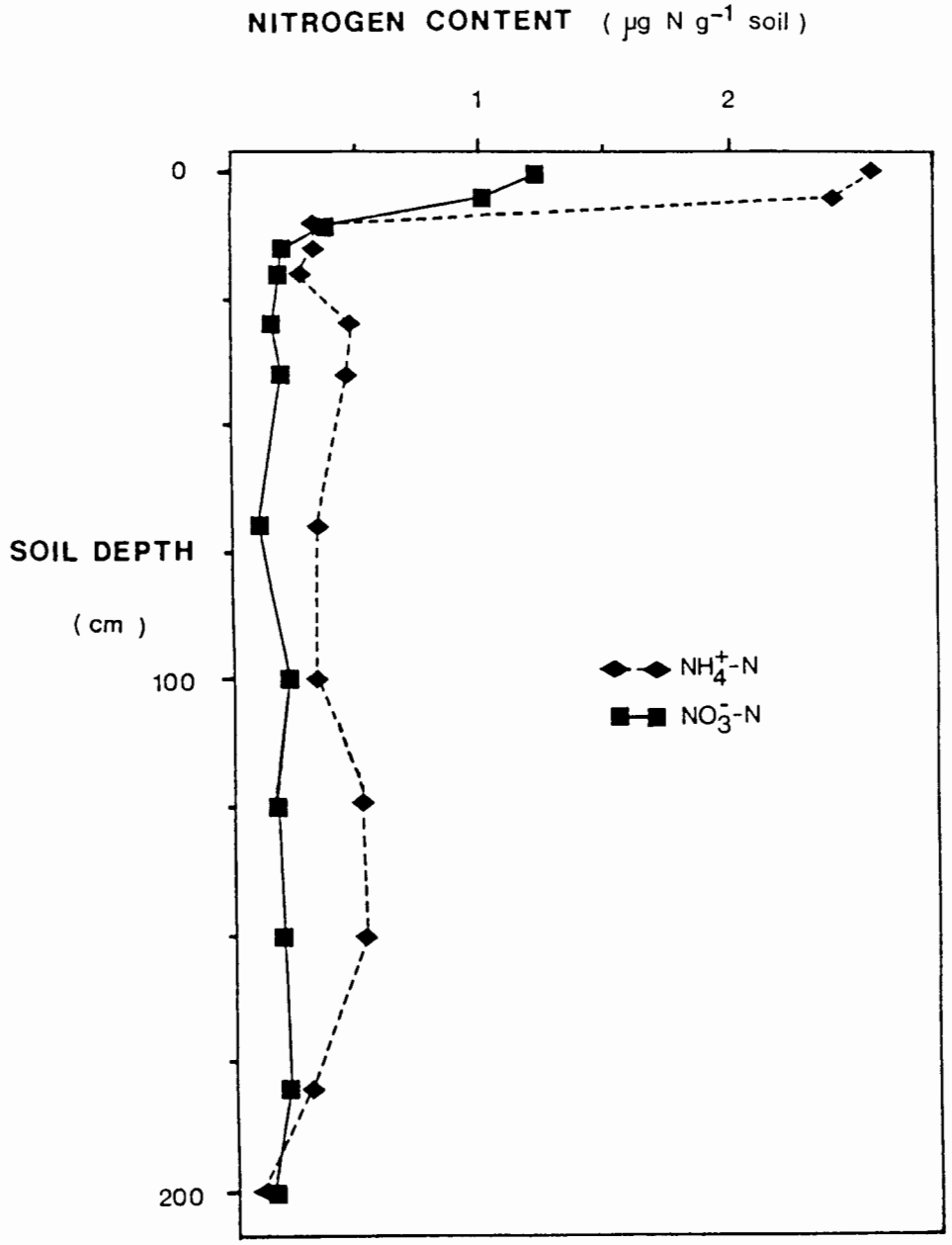


Figure 4.2: The vertical distribution of nitrate and ammonium (µg N g^{-1} soil) to a depth of 200cm in a Clovelly soil form beneath coastal fynbos at Pella, South Africa. Oneway ANOVA showed that both NO_3-N and NH_4-N concentrations (log transformations) were significantly different with soil depth ($F = 2,95$; $df = 12,69$; $p < 0,01$ and $F = 2,77$; $df = 12,69$; $p < 0,01$ for NH_4-N and NO_3-N respectively).

TABLE 4.1: The concentration of total N, NO_3-N and NH_4-N in the rhizosphere region of selected fynbos species growing on a sandy Clovelly soil at Pella, South Africa. Total N, NO_3-N and NH_4-N are expressed as $\mu g\ N\ g^{-1}$ dry soil mass and the values in parentheses are log transformations (Mean ±SD, n=6) used for one-way analysis of variance calculations.

	Nitrogen Concentration ($\mu g\ N\ g^{-1}$ dm)		
	Total N	NO_3 -N	NH_4 -N
Bare Patches	290±67	1.0 [-0.06±0.19]	2.9 [0.41±0.24]
Leucospermum parile	319±58	1.4 [0.08±0.24]	2.1 [0.32±0.08]
Protea repens	259±71	0.9 [-0.05±0.09]	1.3 [0.10±0.09]
Staberhoa distachya	321±54	0.6 [-0.25±0.19]	1.8 [0.19±0.14]
Phylica cephalantha	259±47	1.6 [0.17±0.22]	2.1 [0.27±0.21]
F	1.54	4.02	3.19
d.f.	4.25	4.25	4.23
P	N.S.	0.01	0.05

beneath the different species (Table 4.1) with greater quantities of mineral N found in the soil beneath the bare patches and the soils of the root zones of Phylica cephalantha and Leucospermum parile.

Seasonal variation of soil nitrogen

Changes of total N, NO_3-N and exchangeable NH_4-N concentration in relation to season were studied at various depth intervals down to 100 cm. Total N concentrations showed no variation between month of sampling in the 0 to 5 cm layers and all layers from 30 cm to 200 cm (Fig. 4.3a). In the 10, 15 and 20 cm layers significant ($p < 0,01$) increases in total N during the autumn month of March and he winter month of August were observed. Lowest total N concentrations in the 10 to 20 cm layers were recorded in the summer month of January and autumn month of May.

Soil exchangeable NH_4-N showed significant ($p < 0,05$) monthly variation in the surface to 20 cm layers with the highest concentrations recorded in summer (February, Fig. 4.4a). The lowest exchangeable NH_4-N concentrations were found during August, at the end of winter (Fig. 4.4a). Significant monthly variations ($p < 0,05$) in nitrate concentrations in the surface to 15 cm layers were apparent with no significant changes deeper in the profile. Lowest NO_3-N concentrations occurred during summer (December) with the highest level of NO_3-N recorded during autumn and spring (March and October, Fig. 4.5a).

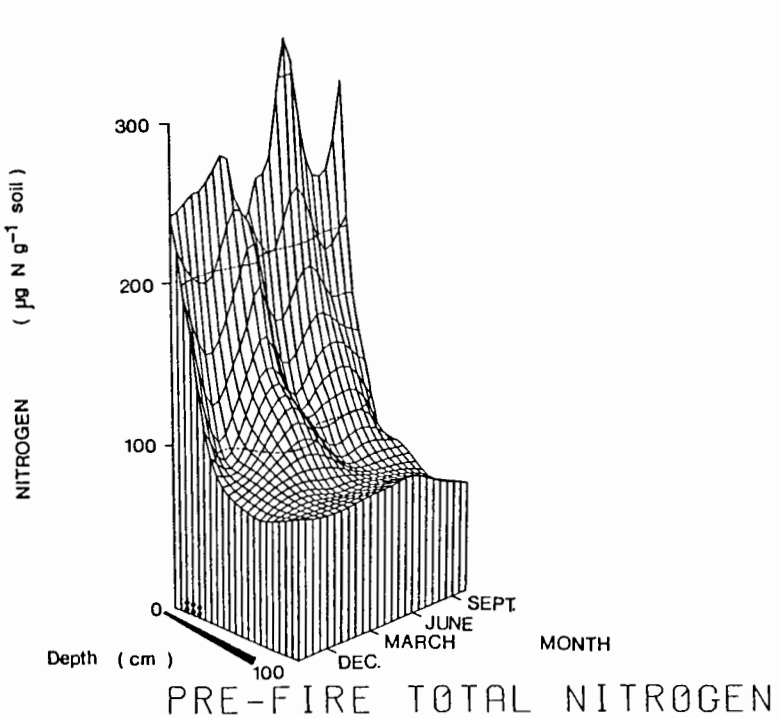


Figure 4.3a: Seasonal variations in soil total nitrogen concentrations to a depth of 100cm beneath a mature 20 year old coastal fynbos stand at Pella, South Africa. Statistically significant monthly variations occur at the depths indicated (●●, $p < 0,01$: ●, $p < 0,05$).

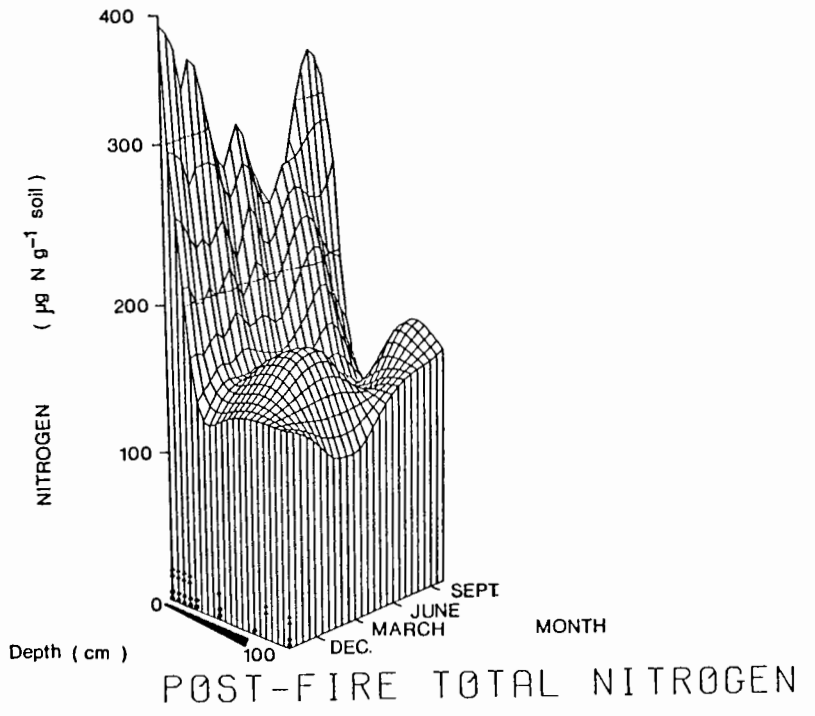
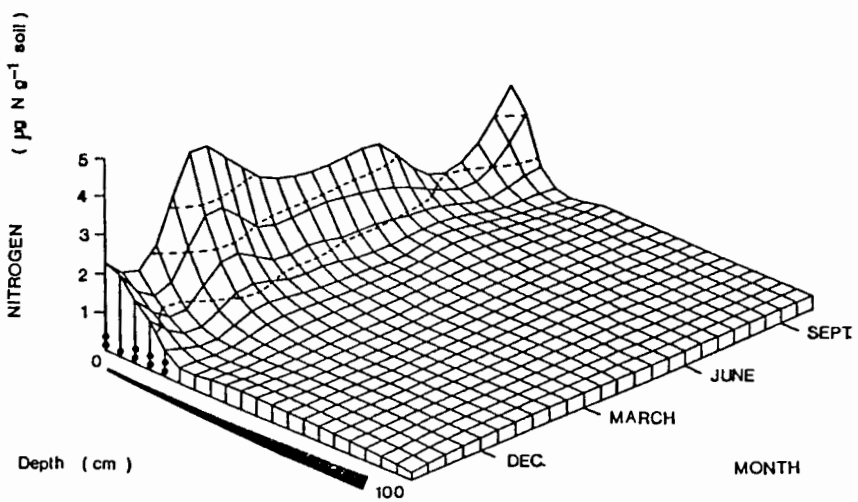
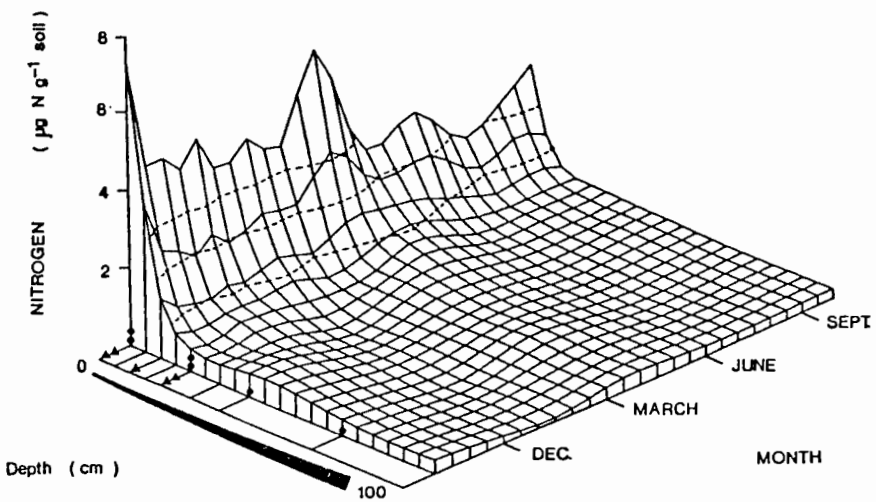


Figure 4.3b: Seasonal variations in soil total nitrogen concentrations to a depth of 100cm in the year following fire destruction of a mature fynbos stand at Pella, South Africa. Statistically significant monthly variations occur at the depths indicated (●●, $p < 0,01$: ● , $p < 0,05$). The effect of fire was also significant at the depths shown (▲▲, $p < 0,01$: ▲ , $p < 0,05$).



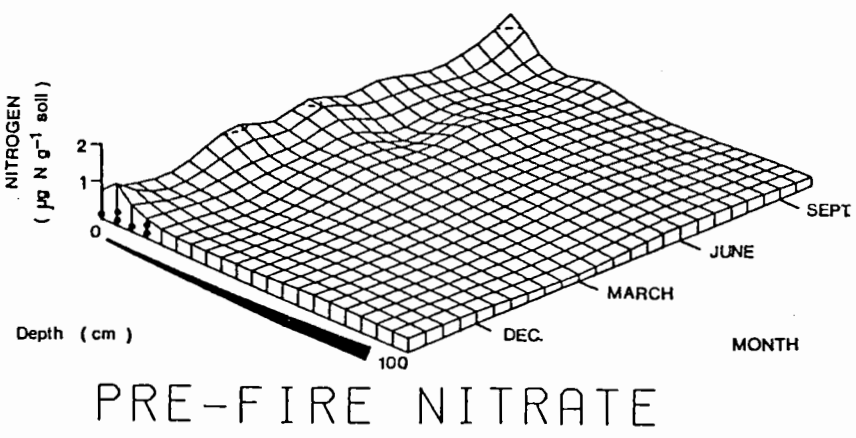
PRE-FIRE AMMONIUM

Figure 4.4a: Seasonal variations in soil exchangeable ammonium concentrations to a depth of 100cm beneath a mature 20 year old coastal fynbos stand at Pella, South Africa. Statistically significant monthly variations occur at the depths indicated (••, $p < 0,01$: •, $p < 0,05$).



POST-FIRE AMMONIUM

Figure 4.4b: Seasonal variations in soil exchangeable ammonium concentrations to a depth of 100cm in the year following fire destruction of a mature fynbos stand at Pella, South Africa. Statistically significant monthly variations occur at the depths indicated (••, $p < 0,01$: •, $p < 0,05$). The effect of fire was also significant at the depths shown (▲▲, $p < 0,01$: ▲, $p < 0,05$).



PRE-FIRE NITRATE

Figure 4.5a: Seasonal variations in soil nitrate concentrations to a depth of 100cm beneath a mature 20 year old coastal fynbos stand at Pella, South Africa. Statistically significant monthly variations occur at the depths indicated (●●, $p < 0,01$: ●, $p < 0,05$).

POST-FIRE NITRATE

Figure 4.5b: Seasonal variations in soil nitrate concentrations to a depth of 100cm in the year following fire destruction of a mature fynbos stand at Pella, South Africa. Statistically significant monthly variations occur at the depths indicated (●●, $p < 0,01$: ●, $p < 0,05$). The effect of fire was also significant at the depths shown (▲▲, $p < 0,01$: ▲, $p < 0,05$).

The effect of fire on soil nitrogen

Soil total N concentration and content increased significantly ($p < 0,05$) throughout the 200 cm profile after fire (Fig. 4.3b) when an input of 66 kg N ha^{-1} from ash deposition was found. In the months following the fire, surface total N concentration decreased from 372 μg N g^{-1} soil immediately after the fire (November 1980) to 283 μg N g^{-1} soil in July 1981. Significant monthly variations ($p < 0,05$) in total soil N occurred during the post-fire period (Fig. 4.3b) and showed a similar seasonal pattern to the pre-fire total N changes (Fig 4.3a) with lowest total N concentrations found in May (autumn).

Soil exchangeable NH_4-N concentrations showed significant changes at only 3 depths (0, 10 and 20 cm) as a result of fire. An ammonium flush at the surface was detected immediately after the fire (November 1980, Fig. 4.4b) and was of a short duration. Monthly variations in exchangeable NH_4-N were only significant at the 0, 20, 40 and 70 cm depths in the post-fire soil.

A flush of soil NO_3-N occurred after the fire (Fig. 4.5b) and the effect of fire was significant ($p < 0,05$) on NO_3-N concentrations in the 0 to 20 cm layers. Below 30 cm no significant changes in NO_3-N levels as a result of fire were found except at 100 cm. Nitrate concentrations in the post-fire soil varied significantly between months at depths from 0 to 40 cm, with the highest NO_3-N concentrations found in the wet autumn and winter months from March to August (Fig. 4.5b).

DISCUSSION

The results indicate that low concentrations of exchangeable NH_4-N and NO_3-N are associated with low total N contents and this may be attributed to climatic influences as well as the parent material of the soil. Campbell (1983) has shown that total soil N is strongly positively correlated with rainfall in montane fynbos while Harradine and Jenny (1958) have shown a positive correlation between total N and clay content in other ecosystems. Thus low rainfall, high temperatures and low clay content have all probably contributed to the low total N content of the soils at Pella.

Spatial heterogeneity of soil nitrogen

Charley and West (1975) proposed that concentration gradients of nitrogen, carbon and organic phosphorus in the soil are the most sensitive indicators of biological function in both vertical and lateral planes. The soil at Pella showed distinct vertical gradients of decreasing quantities of organic matter, total N, exchangeable NH_4-N and NO_3-N with depth. Futher, the dependence of nitrogen, carbon and organic phosphorus distribution on biological processes was demonstrated by the close linear relationship between vertical organic matter distribution and total N concentration in the surface 100 cm of the profile (Fig. 4.1). In the same Clovelly soil form at Pella, Mitchell *et al*. (1984) have shown a decrease in organic phosphorus with depth. Lateral differences in total N concentration were not apparent between the soils of the bare patches and the soils of the rhizosphere regions of the plants

studied. However, lateral differences in NH_4-N and NO_3-N concentrations were significant and probably resulted from N utilization patterns and soil micro-climates differing beneath the species studied. The higher mineral N concentrations found in the soils beneath bare patches and beneath Phylica cephalantha and Leucospermum parile reflected the sparse or absent canopy which exposed the soil to rapid changes of environmental factors which control the mineralization process.

Seasonal variation of soil nitrogen

The monthly variation in total N concentration in soils beneath mature coastal fynbos did not appear to result from increased organic matter input or decomposition at the surface as the only significant differences in soil N content were found in the 10 to 20 cm layers (Fig. 4.3b). Significant monthly variations in mineral N concentrations (NH_4-N plus NO_3-N, Figs 4.4a & 4.5a) were only evident in the surface layers down to 20 cm. These differences probably arose because of better aeration and more pronounced seasonal changes in the surface layers. In the deeper layers of the profile (30-200 cm) which were less influenced by climate, few significant changes in NH_4-N and NO_3-N were found (Figs 4.4a & 4.5a). Most changes at these levels are thought to result from the movement of water within the profile (Gupta & Rorison 1975).

Concentrations of soil exchangeable NH_4-N were low but nevertheless showed a definite seasonal variation in the surface to 20 cm layers. These changes occurred as NH_4-N was either

absorbed by new roots or rapidly nitrified as the microbial population responded to increased soil moisture. A different seasonal pattern of NH_4-N accumulation was found by Lossaint (1973) in soils beneath Quercus ilex at La Madeleine. In this ecosystem, found in the mediterranean-climate zone of France, NH_4-N accumulated only during very cold periods at the end of winter.

Soil NO_3-N concentrations were low and significant monthly variations ($p < 0.05$) were found in the surface to 15 cm layers. Maximum concentrations of NO_3-N occurred in autumn and spring when, as Schaefer (1973) suggested, environmental conditions in a mediterranean-climate zone are optimal for enhanced rates of nitrification. Lowest concentrations of NO_3-N were found in summer. This finding does not agree with the results of Lossaint (1973) who drew attention to high summer NO_3-N levels (maximum of 125 $\mu g\ N\ g^{-1}$ soil) in soils beneath Quercus ilex at the La Madeleine site, France. He attributed the accumulation of NO_3-N to increased nitrification resulting from high temperatures and absence of leaching due to rainfall as the loamy- clay textured soils gradually dried out. The different results obtained in the two mediterranean-climate zones probably reflects the contrasting moisture regimes resulting from the widely different soil textures of the two regions. The low concentrations of mineral N and small seasonal changes found in our investigations appear to be a feature of N limited, undisturbed natural ecosystems.

The effect of fire on soil nitrogen

Direct effects of fire on soil N were manifested as increased total N and NH_4-N concentrations detected at the surface immediately after fire (Figs 4.3b & 4.4b). Of the total above-ground phytomass (39 274 kg ha^{-1}) it was estimated that 76% was consumed by the fire. The extent of N lost by volatilization was uncertain but it was found that 66 kg ha^{-1} were added to the soil by ash deposition. In the Californian mediterranean-climate zone a small increase in soil total N was reported by Christensen & Muller (1975) who estimated that 21 kg ha^{-1} were added to the soil surface after fire in a 40 year Adenostoma chaparral stand. These authors also found that both soil NH_4-N and NO_3-N increased after fire.

Significant increases in exchangeable NH_4-N at the soil surface (Fig. 4.4b) at Pella resulted from the deposition of partially combusted organic N compounds from the above-ground phytomass and from the physiochemical breakdown of organic matter in the soil layers directly affected by the heat of the fire (Russel et al. 1974). Our results show that, as with laboratory burning experiments of DeBano et al. (1979), there was no direct influence of fire on soil NO_3-N levels (Figs 4.5a & 4.5b).

Indirect effects of fire on soil N form, distribution and concentration were apparent in the year following the fire. Higher total N concentrations were found at greater depths in the profile as the year progressed. The total N increases deeper down the profile corresponded with decreases at the surface, probably as a result of leaching of organic N products rendered

soluble by thermal degredation of complex organic N compounds.

The surface postfire NH_4-N flush was temporary and rapidly disappeared (Fig. 4.4b) and no futher influence of fire on NH_4-N concentrations was apparent for the remainder of the year. It appeared that the low post-fire NH_4-N concentrations resulted from its rapid nitrification, because significantly higher ($p<0,05$) soil NO_3-N concentrations were found in the post-fire soil than in the pre-fire soil (Figs 4.5a & 4.5b). Christensen (1973) has also reported high NO_3-N concentrations following a chaparral fire and he attributed the increases to the destruction of nitrification inhibitors by fire. Lewis (1974) suggested that a slow increase in NO_3-N following the fire was in accord with increased microbial activity resulting from direct and indirect effects of the fire on soil properties. In addition to an enhanced bacterial nitrifier population the NO_3-N flush may result from a decreased NO_3-N demand because of the destruction of the mature community. Although fynbos plants are not thought to be efficient at utilizing large quantities of NO_3-N there are species, eg: Protea repens, which can assimilate the small quantities of NO_3-N available in soils beneath mature fynbos stands to help satisfy their low physiological requirement for nitrogen (Stock & Lewis 1984a).

The duration of the post-fire available NO_3-N flush (9 months) was similar to that reported by Christensen (1973) after a chaparral fire although the magnitude of NO_3-N concentration increase was small (1-2 $\mu g\ N\ g^{-1}$ soil) when

compared with his results (20-25 μg N d^{-1} soil). Leaching of NO_3-N down the profile occurred during the winter months possibly adding to the N losses from the ecosystem already incurred from volatilization. Van Wyk (1982) studied the mineral content of drainage waters in a mountain fynbos catchment area after fire and, contrary to our evidence, he found no increased leaching losses of NO_3-N due to fire.

The results of the study show that fire is an effective mineralizing agent in coastal fynbos vegetation as in this study it was shown to have a substantial effect on the concentration, form and distribution of the low nitrogen reserves in the soils beneath such vegetation. When adopting a controlled burning policy for the management of fynbos ecosystems, it is essential that the effects of fire in respect of plant nutrition be taken into account in addition to assessments of the likely damage, community changes and alterations in the physical environment which may be brought about by burning.

ACKNOWLEDGEMENTS

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CHAPTER 5

SOIL NITROGEN MINERALIZATION IN A POST-FIRE COASTAL FYNBOS SUCCESSION AT PELLA, SOUTH WESTERN CAPE, SOUTH AFRICA.

W.D. STOCK, O.A.M. LEWIS and N. ALLSOPP
Botany Department, University of Cape Town,
Rondebosch, 7700, South Africa.

Since the rate of nutrient release often limits the primary productivity of an ecosystem it is of fundamental importance to understand the major processes involved. The mineralization of organic N has been studied in soils of a coastal fynbos ecosystem and the results are reported in this chapter. The study concentrated upon the importance of ecosystem successional age, fire and environmental factors in controlling the mineralization process. The overall mineralization process and stages thereof, namely the nitrification and ammonification processes, were studied in the field and under controlled laboratory conditions using an incubation technique. This chapter is 80% the work of the senior author and it has been submitted for publication as a paper to Oecologia.

INTRODUCTION

Odum (1969) hypothesized that the efficiency of nutrient use in ecosystems intensified as ecological succession proceeded. He suggested that young communities have open nutrient cycles with rapid exchange rates between organisms and environment. As the system matures the cycles become more closed ('less leaky'), the exchanges between the organisms and their environment becoming slower and the role of detritus in nutrient regeneration increasing in importance.

Changes in patterns of nitrogen cycling and more specifically, soil N mineralization associated with succession recently attracted considerable attention when a proposal supporting Odum's hypothesis of "tighter" nutrient cycling in mature communities was provided by Rice and Pancholy (1972). They found that nitrification, progressively decreased in the course of succession in old field forest ecosystems as the soil NO_3-N to NH_4-N ratio decreased and fewer nitrifying organisms were found as the community aged. They suggested that the mechanism causing the decrease was allelochemical inhibition of nitrification (by plant phenolics and their derivatives) and that this was a logical development in the evolution of an ecosystem as it conserves both energy and nitrogen. Where

nitrification occurs slowly, mineral N remains in the relatively immobile NH_4-N form which is retained within the soil by cation exchange. Where the oxidation of NH_4-N is rapid, NO_3-N is produced and is susceptible to loss both by leaching and gaseous pathways. Further, they suggested that energy could be conserved by plants incorporating NH_4-N directly into amino-acids where as NO_3-N assimilation requires reduction steps that are energetically costly.

Support for Rice and Pancholy's proposal has been provided by Rice and Pancholy themselves (1973, 1974), Todd et al. (1975), Lodhi (1979) and Lodhi and Kellingbeck (1980), but subsequent work by Vitousek and Reiners (1975), Vitousek (1977), Lamb (1980), Montes and Christensen (1979) and Robertson and Vitousek (1981) challenged this idea. Workers challenging Rice and Pancholy's (1974) suggestions found that available ammonium was readily oxidized in a variety of communities from several forest seres and that there was no decrease in nitrification with successional age. Vitousek and Reiners (1975) and Vitousek (1977) argued that nitrification occurs throughout the succession and that changes in soil NO_3-N concentration are a result of changes in ecosystem productivity.

The implication of toxins which inhibit nitrification has also been questioned and Purchase

(1974a & b) found no evidence of toxin induced inhibition in Hyparrhenia grasslands in Africa. He ascribed the decrease in nitrification to the lack of available ammonia and other nutrients, in particular phosphorus. Lamb (1980) also showed nitrification to be dependent on the availability of NH_4-N which, in turn, is probably a result of the nutrient stresses that develop in the course of succession.

No studies of nitrification have been undertaken in a fynbos succession but indications of inhibition of nitrification as succession proceeds are evident in the results of Stock and Lewis (1984b) who have shown that NH_4-N to NO_3-N ratios are higher in mature 20 year old coastal fynbos than in regenerating coastal fynbos soils after fire disturbance. In these soils a flush of available NO_3-N, similar to that found by Christensen (1973) in chaparral, was found after fire. Further indirect evidence to support the proposal of Rice and Pancholy (1972) was provided by Stock and Lewis (1984b) who showed that Protea repens L., a late succession species which produces substantial quantities of tannins (Wehmer 1931), utilises NH_4-N preferentially to NO_3-N as its source of nitrogen. The change from NO_3-N to NH_4-N uptake and assimilation during succession has been noted in other ecosystems (Wiltshire 1973, Haines 1977).

The aim of this study was to investigate the

mineralization process in fynbos soils and to ascertain whether or not the hypothesis of Rice and Pancholy (1972) is valid in post-fire succession in coastal fynbos vegetation. This was done by examining the effects of environmental factors, fire and successional age on soil mineralization potential in such a stand.

MATERIALS AND METHODS

Soil Collection

Soil samples were collected from a mature (20 year old) coastal fynbos stand at the Fynbos Biome Intensive Research site at Pella (33^{o} 31'S : 18^{o} 32'E). The soils and vegetation have been described by Mitchell *et al.* (1984) and Stock and Lewis (1984b). Soil samples used to investigate the direct effects of fire on N mineralization were taken at depths of 0-7,5cm on 22nd October 1980 (pre-fire soil) and 5th November 1980 (3 days after a moderately intense fire which completely destroyed the stand). A further 0-1cm deep soil sample was collected on the day of the fire. All samples were analysed, when fresh, for moisture content, pH, exchangeable NH_4-N, NO_3-N and NO_2-N as described below. Thereafter, the soils were air-dried, sieved to remove particles greater than 2mm and stored in plastic bags

before the incubation experiments were undertaken. Total N, oxidizable C and cation content were determined on the air-dried soils as described below.

Samples used to study the N mineralization potential of soils from different seral stages were collected from the surface 0-7,5cm during December 1981 at Pella beneath (1, 6 and 20 year old) Protea repens plants.

The soils were sieved through a 2mm mesh and used immediately for the incubation studies. Sub-samples of these soils were removed for chemical analysis. Moisture content, exchangeable NH_4-N, NO_3-N, NO_2-N, oxidizable C and total N were determined as described below.

Incubation procedures

Incubation experiments to determine the net mineralization potential of pre- and post-fire soils were undertaken in the laboratory using a method modified from Hopmans et al. (1980). The mineral N content (NH_4-N, NO_3-N and NO_2-N) of the air-dried soil sample was determined. Thereafter 20g samples of each soil were placed in 150 ml glass bottles. Water was added to obtain moisture contents in the range from 0, 25, 50, 75, 100 and 150% of field capacity. Field capacity (-0,3 bar) was determined by a pressure plate technique and found to be 2,4% of the soil dry mass

(Fry & Lambrechts, pers comm.*). The incubation bottles were sealed with a polyethylene film which allowed the diffusion of O_2, CO_2 and N_2 but prevented moisture loss (Eno 1960, Gasser 1961). Moisture contents of the samples were, nevertheless, gravimetrically checked each week and, if necessary, water was added to maintain the original moisture content. Samples were incubated in an incubator at 30°C for different periods of time (7, 14, 28 or 42 days), each incubation being quintuplicated.

Samples were extracted with 80 ml 1M KCl and the extracts analysed for mineral N as described below. Potential net mineralization was calculated as the difference between initial mineral N and final mineral N contents.

The investigation of the effect of successional age on potential mineralization was undertaken using a method adapted from Lamb (1980). Soil samples from (0-7,5 cm) beneath 1, 6 and 20 year old P. repens plants were placed in polyethylene bags (200 g). To half the samples 4,8 ml of water was added to ensure that the moisture content was in excess of field capacity, while the rest were maintained at field moisture content. Five replicates of samples from each depth at each site, with

* Fry, M. & Lambrechects, J.J.N., Department of Soil Science, University of Stellenbosch, Stellenbosch, S.A.

water added and without additional water were placed in plastic flower pots containing saturated vermiculite. These were then buried beneath the plant at the depth from which they were removed. A second set of samples was incubated at 30°C in an incubator. After 42 days, 20g of each sample was extracted with 80 ml 1M KCl and the extracts analysed for mineral N as described below.

Soil analysis

Nitrate, nitrite and ammonium in the soil extracts were determined by colorimetric procedures. A copper cadmium reduction of nitrate (Bate & Heelas 1975) followed by nitrite determination using the Griess-Ilosvay method (Jackson 1958) was employed. Nitrite was determined directly by the Griess-Ilosvay method and nitrate by subtraction of nitrite from the Cu/Cd reduction result.

In most cases NO_2-N concentrations were negligible, therefore the separation of NO_2-N values from the NO_3-N and NO_2-N results of the Cu/Cd method was not undertaken. Ammonium was determined by a phenol-hypochlorite procedure modified from Allen et al. (1974) as described by Stock (1983). Total N was determined by Kjeldahl digestion of 2 g air-dried soil samples. Ammonium in the digest was quantified by distillation into 0,02M HCl and subsequent titration

against 0,005M NaOH. All N values were corrected to a concentration on a dry mass basis.

Moisture contents of the soil were determined from mass lost by an 8 g soil sample dried in a forced draught oven at 105°C for 24 hours. Soil pH was determined by suspending 10 g soil in 25 ml of 0,01M $CaCl_2$. After 30 minutes agitation the pH was determined (Scholfield & Taylor 1955). Cation content (K^+, Na^+, Ca^{++} and Mg^{++}) was determined in ammonium acetate soil extracts by flame emission or atomic absorption spectrophotometry. Plant-available phosphorus (Bray No. 2) was determined by the method of Bray and Kurtz (1945).

RESULTS

Changes in soil properties after fire

Total nitrogen concentrations showed significant increases in the 0-7,5 cm soil layer after fire (Table 5.1). Total N increased from 270 to 350 µg N g^{-1} soil. Soil exchangeable NH_4-N also showed an increase from 3,89 to 4,68 µg N g^{-1} soil (Table 5.1) after the fire. Soil organic carbon and cation content (Ca^{++}, Mg^{++}, Na^+ and K^+) did not change markedly after fire. Organic carbon remained at 1,33% while total cation content remained at the pre-fire value (pre-fire sum of cations

TABLE 5.1 : Chemical characteristics of soils beneath coastal fynbos at Pella, South Africa before and after a wildfire (Mean ± SEM).

Soil Description	Soil Nutrient Concentration ($\mu g\ g^{-1}$ soil)											
	Total N ●	Fresh Soil NO_3-N ●	Fresh Soil NH_4-N ●	Stored Soil NO_3-N ●	Stored Soil NH_4-N ●	Ca^{++} ▼	Mg^{++} ▼	Na^{+} ▼	K^{+} ▼	BRAY No 2 P ▼	Organic Carbon (%) *	pH *
Pre-Fire (0-7,5cm)	270 ±14	1,13 ±0,21	3,89 ±0,66	0,77 ±0,03	2,82 ±0,13	104,2	17,0	36,8	19,6	1,28	1,33 ±0,02	4,36 ±0,04
Post-Fire (0-75cm)	350 ±12	1,11 ±0,19	4,68 ±1,16	0,94 ±0,05	4,25 ±0,11	100,2	12,2	39,1	23,5	1,92	1,33 ±0,02	4,79 ±0,04
Post-Fire (0-1cm)	589 ±38	1,02 ±0,13	12,97 ±1,48	1,16 ±0,03	9,29 ±0,47	186,4	26,7	43,7	39,1	5,21	1,87 ±0,01	6,98 ±0,01

▼n=2, * n = 3, ● n = 5

177,6 μg g^{-1} soil while post-fire cation content was 175,0 μg g^{-1} soil). Nitrate values were not influenced by the fire. The distribution of nutrients in the 7,5 cm layer was not constant with depth and higher concentrations of all elements were found in the 0-1cm surface sample (Table 5.1).

Effect of fire on soil nitrogen mineralization

As noted above, post-fire soil contained an appreciably higher N content than pre-fire soil; this remained true for the soils over the whole incubation period of up to 42 days (Figs. 5.1a & b). The amount of inorganic N present in the soil increased with duration of incubation until 28 days, after which with increasing moisture content of the incubated samples an increase in organic N content was found; 100% field capacity appeared to be the optimum soil moisture content for net nitrogen mineralization in this soil type (Figs. 5.1a & b). The rates of net N mineralization under optimum conditions in the pre- and post-fire soils were similar (0,205 and 0,234 μg^{-1} soil d^{-1}; 747 and 677 μg N g^{-1} total nitrogen d^{-1} for the pre- and post-fire soils respectively).

Ammonification and nitrification showed different requirements for optimal activity. The highest ammonium accumulation was found after 14-28 days at moisture

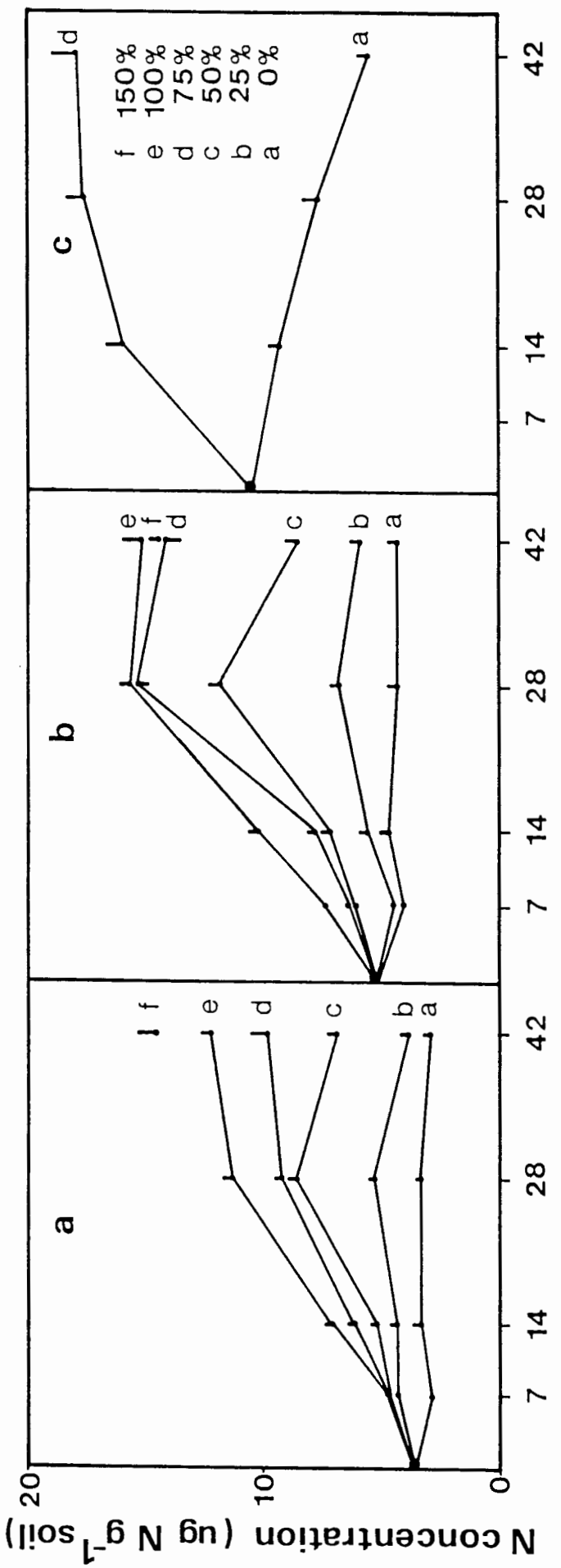

Figure 5.1: The net accumulation of mineral N in (a) pre-fire (0-7,5cm), (b) post-fire (0-7,5cm) and (c) post-fire (0-1cm) soils beneath coastal fynbos vegetation when incubated for 7, 14, 28 or 42 days at moisture contents between 0-150% of field capacity (Mean + or - SEM, n = 5).

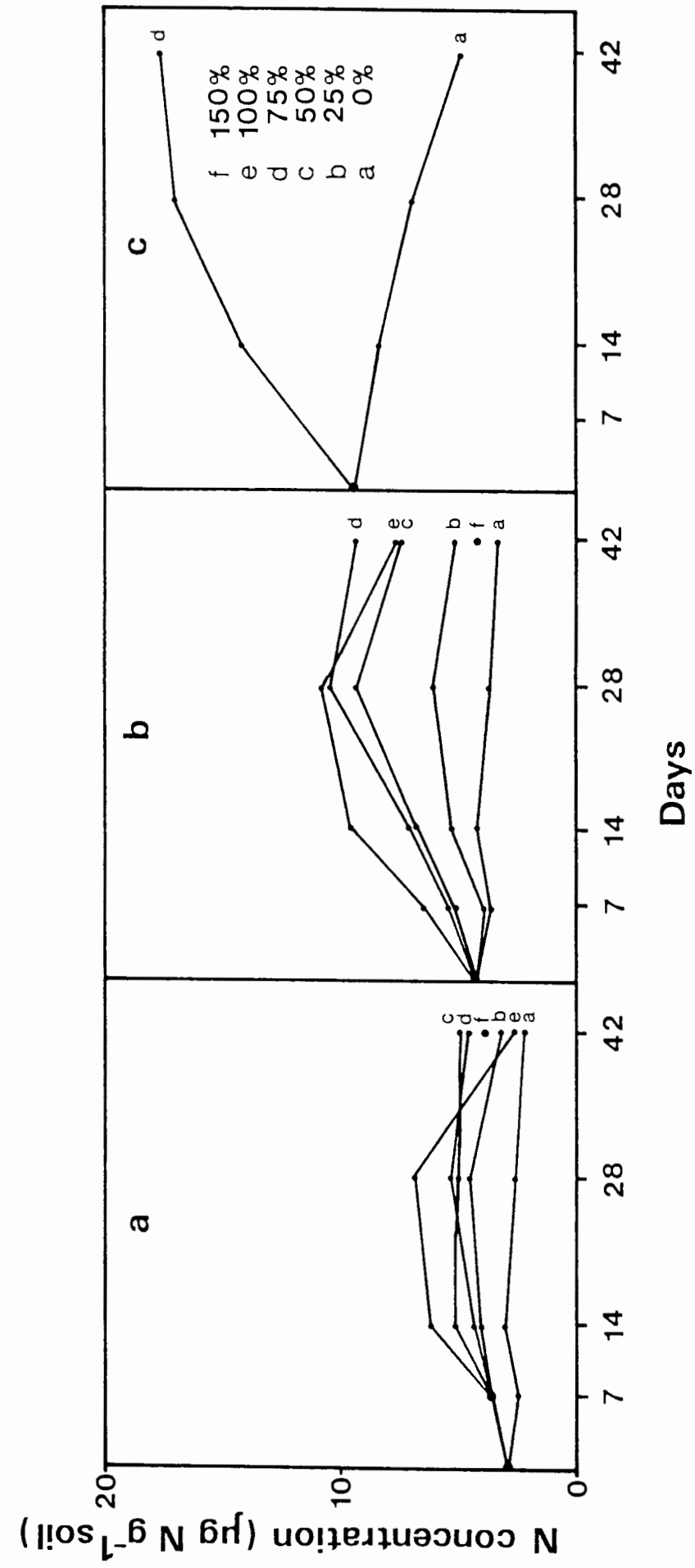

Figure 5.2: The net accumulation of ammonium in (a) pre-fire (0-7,5cm), (b) post-fire (0-7,5cm) and (c) post-fire (0-1cm) soils beneath coastal fynbos vegetation when incubated for 7, 14, 28 or 42 days at moisture contents between 0-150% of field capacity.

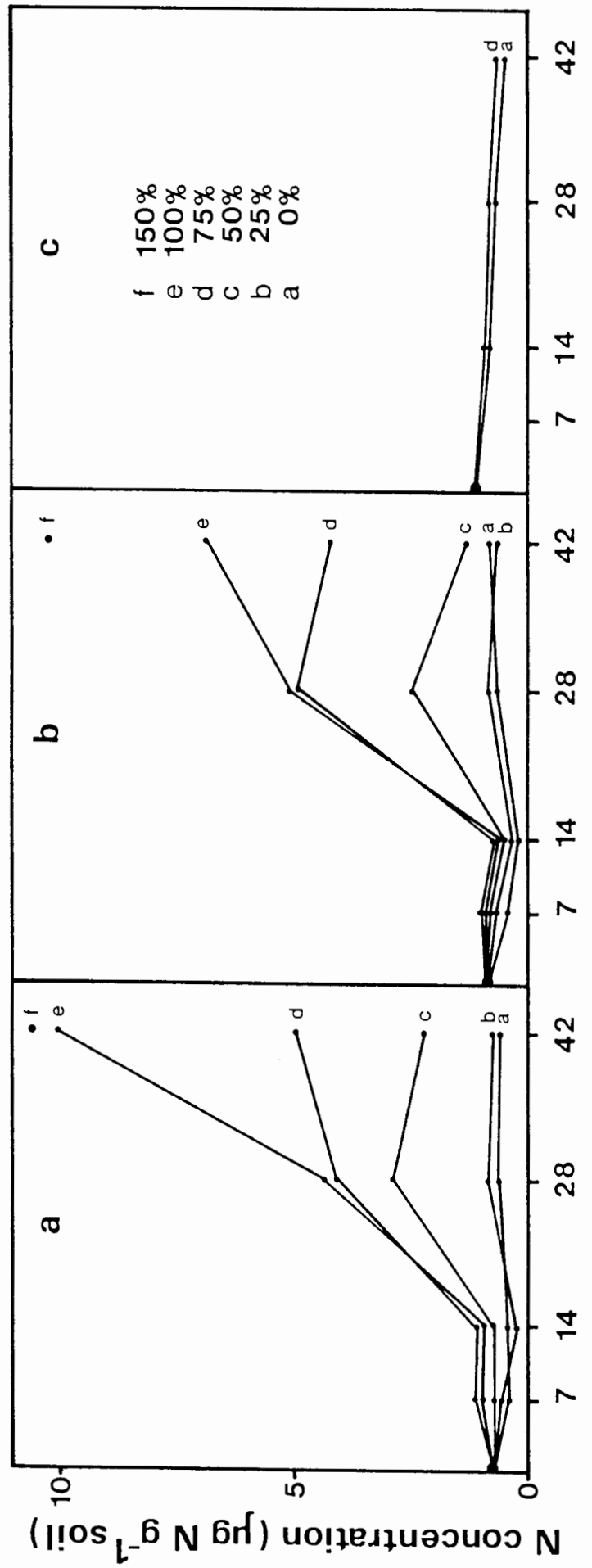

Figure 5.3: The net accumulation of nitrate in (a) pre-fire (0-7,5cm), (b) post-fire (0-7,5cm) and (c) post-fire (0-1cm) soils beneath coastal fynbos vegetation when incubated for 7, 14, 28 or 42 days at moisture contents between 0-150% of field capacity.

contents of 75 and 100% field capacity (Figs. 5.2a, b & c). Ammonium content in post-fire soils was higher initially and after incubation than in the pre-fire soils. The rate of NH_4-N production under optimum conditions was also higher in the post-fire soils than in the pre-fire soil (0,191 and 0,143 µg NH_4-N g^{-1} soil d^{-1} respectively).

No net nitrate accumulation was shown in the first 14 days of incubation (Figs. 5.3a, b & c) and highest levels of nitrate were found after 28-42 days in both the pre- and post-fire soils. Initially NO_3-N levels were similar in both soils. Optimum moisture content for maximum NO_3-N accumulation was 100-150% of field capacity. The post-fire 0-1cm sample showed no net nitrate accumulation (Fig. 5.3c). No differences were found under optimum conditions in rates of nitrate accumulations in pre- and post-fire soils (0,229 and 0,214 µg NO_3-N g^{-1} d^{-1} respectively).

Changes in soil chemical properties during succession

Total N, organic carbon, cations (K^+, Na^+, Ca^{++}, Mg^{++}) and NO_3-N showed decreased concentrations in soils beneath the 6 year old plants as compared to the soils beneath the 1 year old P. repens sampled. The soils beneath the 20 year old P. repens stand had higher concentrations of total N and organic carbon than the

TABLE 5.2: Chemical characteristics of soils sampled from 0 - 7,5cm beneath different successional ages (1,6 and 20 years old) of coastal fynbos at Pella, South Africa (Mean ± SEM).

Stand Age	Soil Nutrient Concentration (µg g^{-1} soil)							Organic Carbon (%)▼	pH*	Moisture Content (%)*
	Total N●	NO_3-N●	NH_4-N●	Ca^{++}▼	Mg^{++}▼	Na^{+}▼	K^{+}▼			
1	274 ±14	3,24 ±0,13	3,27 ±0,32	124,3	23,1	41,4	70,4	1,39	5,06 ±0,01	0,49 ±0,02
6	245 +16	0,25 ±0,01	2,36 ±0,45	102,2	14,6	32,2	27,4	0,72	5,03 ±0,01	0,67 ±0,01
20	454 ±34	1,53 ±0,22	2,13 ±0,52	108,2	18,2	41,4	43,0	1,65	4,98 ±0,03	1,63 ±0,06

▼n=2, *n=3, ●n=5

soils beneath the 1 year old plants (Table 5.2). Other elements such as NO_3-N, K^+, Na^+ Mg^{++} and Ca^{++} were higher in soils beneath the 20 year old P. repens than in soils beneath the 6 year old plants but not as high as beneath the 1 year old plants. Surface pH values remained almost constant during succession (pH 5,06 to 4,98).

Effect of successional age on soil N mineralization

Potential net mineralization increased with successional age (Fig. 5.4a). The rate of inorganic N accumulation increased with successional development when expressed on a soil dry mass basis (0,260, 0,310 and 0,438 µg N g^{-1} d^{-1} for the 1, 6 and 20 year old soils respectively). However this trend was not shown when inorganic N accumulation was calculated on a total N content basis. The 6 year old site showed the highest rate of mineral N accumulation although the rates were quite similar (947, 1244 and 965 µg N g^{-1} total N d^{-1} for the 1, 6 and 20 year old sites respectively). In all cases mineral N accumulation was predominately in the NO_3-N form.

The incubation of samples collected in summer without water added (Fig. 5.4a) showed that moisture limited N mineralization activity. Samples with water added, incubated in the field and at 30°C in the

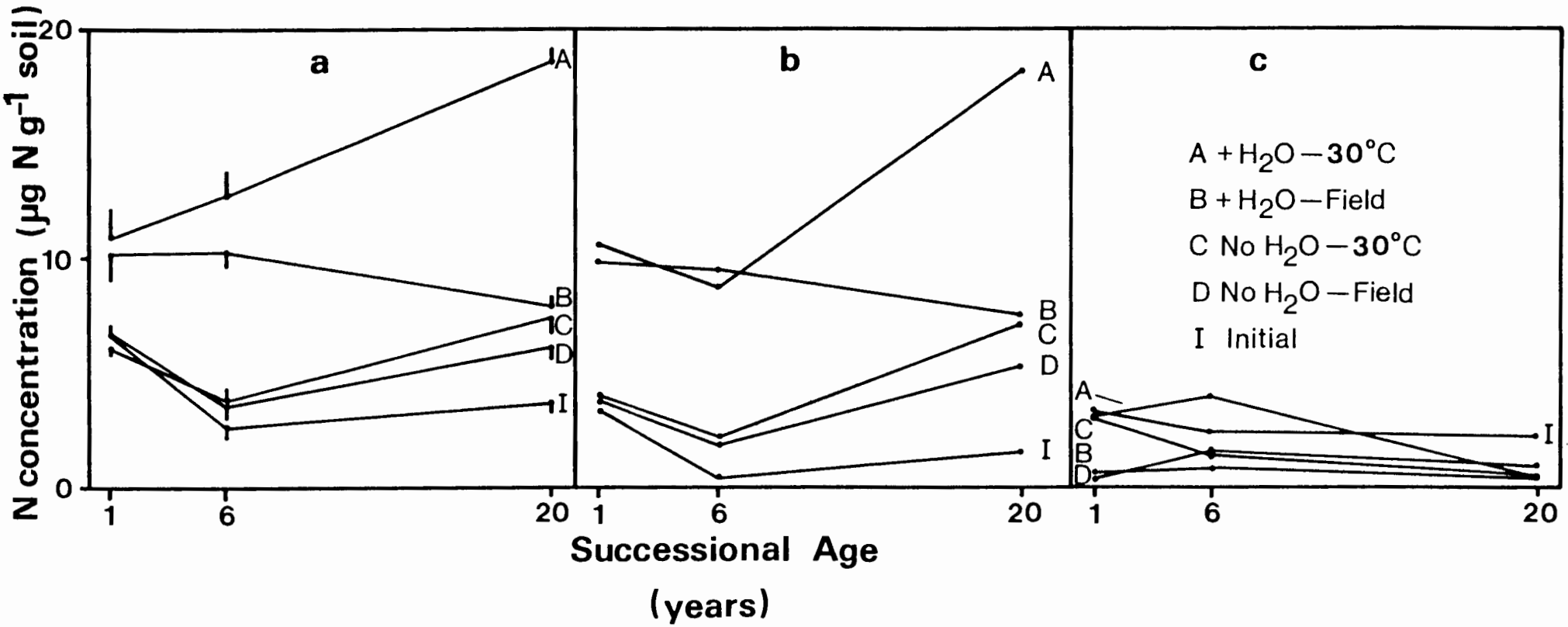

Figure 5.4: The net accumulation of (a) mineral N, (b) nitrate and (c) ammonium in soils 0-7,5cm deep beneath different seral stages of a coastal fynbos succession. Soils were incubated for 42 days at different moisture contents (in situ field moisture content or at field capacity) either in the field or under laboratory conditions (30°C). (Mineral N, Mean ± SEM).

laboratory, showed significant mineralization activity. Although the 20 year old sample had the highest inorganic N accumulation of all succession ages when incubated in the laboratory, it was the lowest when incubated in the field (Fig. 5.4a).

Ammonium accumulation showed no influence from community successional age and ammonium levels were below those found on the soil sampling date (Fig. 5.4c). Net potential nitrification on the other hand was highest in the oldest successional stage and lowest in the soil beneath the 6 year old plants (Fig. 5.4b). Rates of NO_3-N production increased throughout the succession from 625 to 881 $\mu g\ N\ g^{-1}$ total $N\ d^{-1}$). After incubation NO_3-N concentrations always exceeded those found at the initial sampling date during December 1981.

DISCUSSION

The effects of fire on soil N and subsequent microbial transformations of N have been studied in a number of ecosystems, including coastal fynbos (Christensen 1973, Debano *et al*. 1979, Mayland 1967, Stock & Lewis 1984b). In all studies, availability of N was improved immediately after fire as a result of NH_4-N liberated by physiochemical breakdown of organic matter by heat (Table 5.1). Following the NH_4-N increase,

flushes of available NO_3-N have been reported in the first year following fire (Christensen 1973, Stock & Lewis 1984b). These elevated NO_3-N concentrations have been suggested to arise from increased soil nitrification arising from elevated pH values, the removal of microbial nutrient stresses by the liberation of nutrients in the ash (Weber & Gainey 1962, Fowells & Stephenson 1934) and to the heat destruction of allelochemical nitrification inhibitors. Contrary to these suggestions our results showed that although the post-fire soil contained more available N from the physical release of N from the fire, the rates of biochemical inorganic N production (potential net mineralisation rates) were similar for pre- and post-fire soils when assayed using an incubation technique. The post-fire accumulation of NO_3-N beneath coastal fynbos after fire (Stock & Lewis 1984b) therefore appears to have resulted from fire induced changes in the factors controlling the equilibrium between N mineralization, immobilisation, system losses and plant uptake. Our results indicated that changes in soil moisture conditions greatly influenced the N mineralisation process (Figs. 5.1a, b & c) and it has been shown in forest ecosystems that microclimate changes caused by clear cutting, namely higher soil temperatures with adequate moisture, lead to rapid

mineralization and nitrification rates *in situ* (Focht & Verstraete 1977, Matson & Vitsousek 1981). In addition to the influence of the altered microclimate the increased availability of NO_3-N in the field may possibly be a consequence of reduced competition for nutrients. The diminished nutrient requirement of the regenerating biomass as opposed to the higher demand of the larger biomass of the pre-fire mature community may have accounted for the patterns of mineralization and nitrification observed after fire disturbance (Vitousek & Reiners 1975).

A direct influence of fire on soil mineralization was noticed by the complete absence of nitrifier activity in the 0-1cm layer of the soil (Figure 5.2c) due to the 'partial sterilisation' effect (Raison 1979), although ammonification was found to occur in this soil. Results expected from the Rice and Pancholy (1972) suggestion namely, (i) a decrease in NO_3-N production in samples from older seral stages and (ii) that NO_3-N would represent a smaller proportion of net nitrogen mineralization in each successive sere (Robertson & Vitsousek 1981) were not evident.

Potential mineralization increased with successional age while potential nitrification showed no definite trend. Soil of the 20 year old *P. repens* stand had the highest accumulation of NO_3-N (Fig. 5.4b) while the soil

of the 6 year old community showed the lowest NO_3-N production. In all cases most of the N mineralization was in the form of NO_3-N and the ratio of NH_4-N to NO_3-N remained constant in all seral stages (Figs. 5.4b & c). These results confirm the findings of other workers who studied nitrification potentials in soils of secondary successions of different ecosystems (Lamb 1980, Montes & Christensen 1979, Robertson & Vitousek 1981). The results further showed that increased mineralization and nitrification appeared to be associated with increases in soil total N rather than with successional age. This suggests that ammonification releases more NH_4-N which becomes increasingly available to nitrifiers as the total N of the soil increases. Robertson (1984) found similar results in seral stages from a rainforest succession where NO_3-N production consistently reflected total mineral N production. The addition of NH_4-N to these soils further stimulated NO_3-N production and he suggested that nitrification is ammonium limited at all stages of lowland rainforest succession.

However, the dependence of nitrification of NH_4-N availability and the lack of allelochemical inhibition did not account for the <u>in situ</u> patterns of high NH_4-N to NO_3-N ratios found in coastal fynbos soils (Table 5.2 and Stock & Lewis 1984b). In soils incubated in the field the importance of environmental factors on N

mineralization were apparent. The soils collected and incubated *in situ* during the dry summer months without extra water added showed water limitation of the whole mineralization process (Figs. 5.4a, b & c) while the *in situ* samples with water added revealed the influence of microclimate on mineralization. The open canopy of the 1 year and 6 year old stands probably accounted for higher soil temperatures which enabled more mineral-N production to occur than in the 20 year old stand where the dense canopy prevented solar radiation from influencing soil temperatures. It has been shown by these incubation studies that soils beneath fynbos vegetation have the potential to accumulate NO_3-N. However the lag phase of 14 days before nitrification occurs (Fig. 5.3b) suggested that the constant conditions of the incubation procedure without the presence of roots favours the accumulation of mineral-N in the form of NO_3-N. But in the field these sandy, freely draining soils probably never have sufficiently constant moisture and environmental conditions to enable unlimited nitrification to proceed. The high NH_4-N : NO_3-N ratios *in situ* may result from the ammonification process being less sensitive to low water potentials (Griffin 1972) and prevailing temperatures than the nitrification process (Alexander 1977). Therefore, the incubations for 14-28 days (Figs. 5.2a, b & c) showing

NH_4-N accumulation might be a better approximation of dynamic field conditions than the longer 42 days incubation period used.

It may be concluded from the results of this study that there appears to be no increasing allelopathic inhibition of nitrification in the soils of different seral stages of a post-fire coastal fynbos succession. Rather, the production of NO_3-N appears to be related to rates of mineralization and total N content of the soils studied, independent of successional age.

CHAPTER 6

UPTAKE AND ASSIMILATION OF NITRATE AND AMMONIUM BY AN EVERGREEN FYNBOS SHRUB SPECIES *PROTEA REPENS* L. (PROTEACEAE).

W.D. STOCK and O.A.M. LEWIS
Botany Department, University of Cape Town,
Rondebosch, 7700, South Africa.

In a low nutrient environment, such as coastal fynbos ecosystems, plant species must adopt a number of strategies to overcome nutrient limitation. In this chapter and in Chapter 3 (Section 3.2.4) investigations into the N assimilation potential of a characteristic fynbos family (Proteaceae) have been reported. The investigations used enzyme assays and ^{15}N uptake and incorporation studies to describe the ability of these plants to absorb and assimilate different forms of inorganic N. The results of the nitrate reductase enzyme studies have been published as a paper which appeared in the South African Journal of Botany 1 (4): 124-126 (1982). The results reported in this chapter deal with $^{15}NO_3$-N and $^{15}NH_4$-N uptake and assimilation by members of the Proteaceae and this chapter has been published as a paper in the New Phytologist 97: 261-268 (1984).

INTRODUCTION

It is generally agreed that under natural conditions the main nitrogen sources for higher plants are nitrate and ammonium (Haynes & Goh 1978). Research on herbaceous crops which have evolved from ruderal species characteristic of nutrient-rich, disturbed sites has shown that each ion produces a different physiological response within the plant (Cox & Reisenauer 1973, Reisenauer 1978) and that plants differ in their ability to absorb and assimilate nitrate and ammonium (Krajina, Madoc-Jones & Memmor 1973, Lewis, James & Hewitt 1982).

It has been proposed that nitrification in an ecosystem is inhibited by plant phenolics produced as succession proceeds (Rice & Pancholy 1972, 1973), resulting in soils under climax vegetation having a low NO_3-N content and availability. The advanced secondary stages of fynbos vegetation of the South Western Cape Province of South Africa are dominated by members of the Proteaceae which are perennial, evergreen slow growing plants, and we have found soils under mature (29 year old) Protea repens do, indeed, contain greater quantities of NH_4-N than NO_3-N.

Members of this family appear to have adapted to the low nitrogen status of the soils by possessing a low

rate of nitrogen metabolism to correspond with a slow growth habit (Lewis & Stock 1978). They also have a poor NO_3-N assimilation potential as reflected by low activities of nitrate reductase in the shoots and roots (Stock & Lewis 1982), suggesting that members of the Proteaceae are suited to the later successional stages of the fynbos ecosystem by utilizing NH_4-N as the predominant N source in association with a low N assimilation rate.

The objective of this study was to compare the uptake and assimilation of $^{15}NO_3$-N and $^{15}NH_4$-N in Protea repens to establish whether there is a preferred N source for the growth and development of this plant and to investigate the sites and rates of NH_4-N and NO_3-N assimilation in this species.

MATERIALS AND METHODS

Plant Material

Seeds of Protea repens (L.) L. were collected at the Fynbos Biome Research Site at Pella (33° 31'S : 18° 32'E) after a wildfire. The seeds were sown on sand and 14 days after germination the seedlings were transplanted into pots containing acid washed sand. They were grown for 3 months in a well ventilated glasshouse and watered every 2 or 3 days with tap water until the

cotyledons were chlorotic, indicating that all internal nutrient reserves had been utilized. The plants were then watered every 3 days with 50 ml of one-tenth dilution of standard Long Ashton nutrient solution containing a mixture of 0,1mM NO_3-N and 0,1mM NH_4-N (Hewitt 1966). After 14 days on this nutrient regime the ^{14}N was leached out of the acid washed sand in the pots with distilled water. From the following day 9 plants were fed every 2 days with 50 ml of a one-tenth dilution of the Long Ashton solution containing 2mM 99,8 A%E ^{15}N KNO_3 (Prochem Ltd, Deer Park Rd. London, U.K.) at pH 5 and a further 9 plants with the diluted standard Long Ashton solution at pH 5 containing 2mM 99,8 A%E ^{15}N NH_4Cl. The pot leachate from the $^{15}NH_4$-N fed plants was analysed every 2 days to ascertain that no nitrification had occurred. Harvests were made after 3, 6 and 9 days, 3 plants from each feeding regime being sampled at each harvest and ethanol soluble and bound N compounds extracted for determination of ^{15}N enrichment.

Extraction and preparation of samples for ^{15}N determination.

On harvesting, the fresh mass of the plant organs was determined and the material killed in liquid nitrogen, homogenized in cold 80% (v/v) ethanol (1 g fresh mass in 50 ml ethanol) and ethanol-soluble N

compounds allowed to extract for 24 h at 0°C. The samples were then filtered through Whatman No 1 filter paper. The filtrate containing the ethanol-soluble N was evaporated down to 10 ml under an airstream while the residue remaining on the filter paper was oven dried at 80°C prior to micro-Kjeldahl digestion to determine the quantity of bound N, and ^{15}N enrichment of the fraction.

The preparation of soluble N samples for ^{15}N atomic emission spectrophotometric analysis differed for NO_3-N and NH_4-N fed plants, and was as follows:-
The 10 ml ethanol-soluble N fraction from the NO_3-N fed plants was passed through a 3 x 1 cm ion exchange column ('Dowex' 50W-X8 Standard H^+, 100-200 mesh particle size, Dow Chemical Company, Michigan, USA) to separate NO_3-N from the organic + ammonium fraction as described by Atkins and Canvin (1971). The nitrate fraction was placed in a Markham semi-micro distillation unit, 0,3 g Devarda's Alloy and 0,2 g heavy MgO powder (previously heated to 600°C for 2 h and cooled) added and the ammonia produced after steam distillation collected in 2 ml 0,02M HCl and back titrated with 0,005M NaOH using Tshiro's indicator (screened methyl red). The organic + ammonium fraction of the nitrate fed plants was converted to ammonium by micro-Kjeldahl digestion with 3 ml conc. N-free sulphuric acid and a selenium catalyst tablet followed by steam distillation with 12 ml 50%

(w/v) NaOH. The ammonium was collected in 2 ml 0,02M HCl and prepared for ^{15}N analysis as described above.

Ammonium in the ethanol-soluble sample of the NH_4-N fed plants was separated from the organic N fraction by a 3 min distillation with 0,2 g MgO as the alkalizing agent. This short distillation with mild alkali prevented hydrolysis of the amides in the organic N fraction (Bremner 1965a). The distillate was collected and its ammonium content determined by titration.

The organic N fraction of the NH_4-N fed plants was prepared for ^{15}N analysis as described for the NO_3-N fed plants. Each fraction converted into the ammonium form was reduced in volume to $\pm$ 1 ml and aliquots oxidised with sodium hypobromite under vacuum to produce N_2-filled discharge tubes by the method of Faust (1967). ^{15}N enrichment was determined on a Statron NOI 4 atomic emission spectrophotometer (Packard Instruments International S.A., Zurich, Switzerland) and the results calculated from a calibration curve constructed for standards between 0,5% and 50% ^{15}N enrichment. Bound N was determined after micro-Kjeldahl digestion (followed by steam distillation) of the ethanol-soluble residues, ^{15}N enrichment being estimated on the concentrated distillate as described above.

RESULTS

Uptake and assimilation of nitrate and ammonium.

Young plants of Protea repens exhibited low nitrogen uptake and assimilation rates for both NO_3-N and NH_4-N feeding (4,8 µg $^{15}N\ g^{-1}$ fm d^{-1} for NO_3-N fed plants and 5,9 µg $^{15}N\ g^{-1}$ fm d^{-1} for NH_4-N fed plants, Table 6.1) when compared with uptake and assimilation rates of rapidly growing species from more fertile habitats (eg: barley with an assimilation rate of 12 µg $^{15}N\ g^{-1}$ fm h^{-1} and 39 µg $^{15}N\ g^{-1}$ fm h^{-1} respectively for 2mM hydroponically grown NO_3-N and NH_4-N plants, Lewis & Chadwick 1983).

While the uptake rates for both ^{15}N forms was low, the total quantity of $^{15}NH_4$-N (50,2 µg $^{15}N\ g^{-1}$ fm) absorbed and assimilated by P. repens over 9 days was greater than $^{15}NO_3$-N uptake and assimilation (33,4 µg $^{15}N\ g^{-1}$ fm) over the same time period (Table 6.1).

Internal distribution of total nitrogen.

The mean N content for the 18 experimental plants was 2964 µg N $plant^{-1}$ with a mean N concentration of 896,2 µg N g^{-1} fresh mass. The distribution pattern of nitrogen between root and shoot was the same for all plants irrespective of the N form in the feeding solution for the duration of feeding. The root contained

TABLE 6.1: Total nitrogen concentration, ^{15}N concentration, ^{15}N uptake and assimilation rate and plant fresh mass of young plants of Protea repens after 3, 6 and 9 days feeding with either 2mM NO_3-N or 2mM NH_4-N (Mean of 3 plants ± SEM).

	Nitrogen source 2mM $^{15}NH_4^+$			Nitrogen source 2mM $^{15}NO_3^-$		
Duration of feeding period (days)	3	6	9	3	6	9
N concentration (μg N g^{-1} fresh mass)	900,1 ±136,00	911,6 ± 46,8	868,6 ± 30,4	975,4 ±100,3	835,2 ± 44,8	886,3 ± 32,4
^{15}N concentration (μg ^{15}N g^{-1} fresh mass)	19,0 ± 1,9	34,8 ± 2,9	50,2 ± 8,4	19,3 ± 2,4	25,0 ± 6,5	33,4 ± 9,7
^{15}N uptake and assimilation rate (μg ^{15}N g^{-1} fresh mass d^{-1})	6,3 ± 0,6	5,8 ± 0,5	5,6 ± 0,9	6,4 ± 0,8	4,2 ± 1,1	3,7 ± 1,1
Plant fresh mass (g)	3,40 ± 0,32	3,78 ± 0,40	3,92 ± 0,06	3,53 ± 0,34	3,24 ± 0,29	3,62 ± 0,27

TABLE 6.2: ^{15}N enrichment, ^{15}N content in each plant part and ^{15}N concentration in the ethanol soluble fraction of the roots and shoots of young plants of Protea repens after 3, 6 and 9 days feeding with either 2mM NO_3-N or 2mM NH_4-N (Mean 3 plants ± SEM).

		Nitrogen source 2mM $^{15}NH_4^+$			Nitrogen source 2mM $^{15}NO_3^-$		
Duration of feeding period (days)		3	6	9	3	6	9
ROOT	Enrichment A%E	8,3 ±0,2	6,4 ±0,3	14,0 ±1,2	4,5 ±0,7	5,4 ±0,9	,6,5 ±1,4
	^{15}N Content (μg ^{15}N per plant part)	15,1 ±1,9	16,7 ±1,8	27,3 ±3,9	8,5 ±1,1	6,4 ±1,0	5,9 ±1,4
	^{15}N Concentration (μg ^{15}N g^{-1} f.m.)	8,5 ±1,5	9,8 ±1,2	14,0 ±1,7	4,9 ±0,2	4,5 ±1,2	3,8 ±0,3
SHOOT	Enrichment A%E	2,1 ±0,6	3,0 ±0,6	3,9 ±0,8	3,9 ±1,0	3,3 ±1,0	2,6 ±0,8
	^{15}N Content (μg ^{15}N per plant part)	5,0 ±1,7	8,3 ±2,2	8,6 ±1,7	8,1 ±0,5	4,6 ±1,4	3,6 ±1,3
	^{15}N Concentration (μg ^{15}N g^{-1} f.m.)	3,2 ±1,1	3,8 ±0,7	4,3 ±0,7	4,5 ±0,8	2,8 ±1,0	1,6 ±0,5

TABLE 6.3: ^{15}N enrichment, ^{15}N content in each plant part and ^{15}N concentration in the bound N fraction of the roots and shoots of young plants of *Protea repens* after 3, 6 and 9 days feeding with either 2mM NO_3-N or 2mM NH_4-N (Mean of 3 plants ± SEM).

		Nitrogen source 2mM $^{15}NH_4^+$			Nitrogen source 2mM $^{15}NO_3^-$		
Duration of feeding period (days)		3	6	9	3	6	9
ROOT	Enrichment A%E	4,3 ±0,6	8,6 ±1,2	11,5 ±0,9	4,9 ±0,5	5,9 ±1,0	9,6 ±2,9
	^{15}N Content (µg ^{15}N per plant part)	32,3 ±1,9	73,2 ±15,8	101,7 ±14,1	35,6 ±2,7	44,4 ±7,9	88,8 ±20,6
	^{15}N Concentration (µg ^{15}N g^{-1} f.m.)	18,3 ±2,6	43,3 ±9,4	52,2 ±6,1	21,7 ±4,9	30,8 ±7,6	62,4 ±14,7
SHOOT	Enrichment A%E	0,6 ±0,1	1,5 ±0,3	2,7 ±0,5	0,7 ±0,1	1,3 ±0,4	1,2 ±0,3
	^{15}N Content (µg ^{15}N per plant part)	11,3 ±2,2	31,9 ±8,4	59,3 ±16,5	14,1 ±4,2	22,6 ±7,5	25,0 ±8,6
	^{15}N Concentration (µg ^{15}N g^{-1} f.m.)	7,2 ±1,4	15,7 ±3,6	29,8 ±8,2	7,7 ±1,7	13,5 ±4,6	11,2 ±3,5

31% (Range 27,3 to 32,4%) of the total plant N with the balance, 69% (Range 67,6 to 72,7%), contained in the shoot (Fig. 6.1).

The fresh mass of the plants fed on the different N sources (Table 6.1) did not change over the short duration feeding period and no mass differences could be ascribed to either NO_3-N or NH_4-N nutrition.

Sites of ^{15}N incorporation and accumulation.

After 9 days the ^{15}N content of the $^{15}NH_4$-N fed plants increased to a maximum of 197,0 µg ^{15}N per plant which constituted 5% of total plant N. ^{15}N content of the $^{15}NO_3$-N fed plants did not increase to the same extent and reached a maximum of 123,4 µg ^{15}N per plant (3,9% of total plant N) (Table 6.1.).

^{15}N distribution between the soluble and bound fractions within the roots and shoots followed a similar pattern for both forms of N feeding with approximately 70% of the ^{15}N incorporated in the root (Fig. 6.1). The major ^{15}N containing fraction in the root was bound N which reached enrichment levels of 11,5 A%E ^{15}N and 9,6 A%E ^{15}N after 9 days for the $^{15}NH_4$ and $^{15}NO_3$-N fed plants respectively (Table 6.3). Bound ^{15}N in the shoots only attained maximum values of 2,7 A%E ^{15}N and 1,2 A%E ^{15}N respectively for the $^{15}NH_4$-N and $^{15}NO_3$-N fed plants over the same time period (Table 6.3).

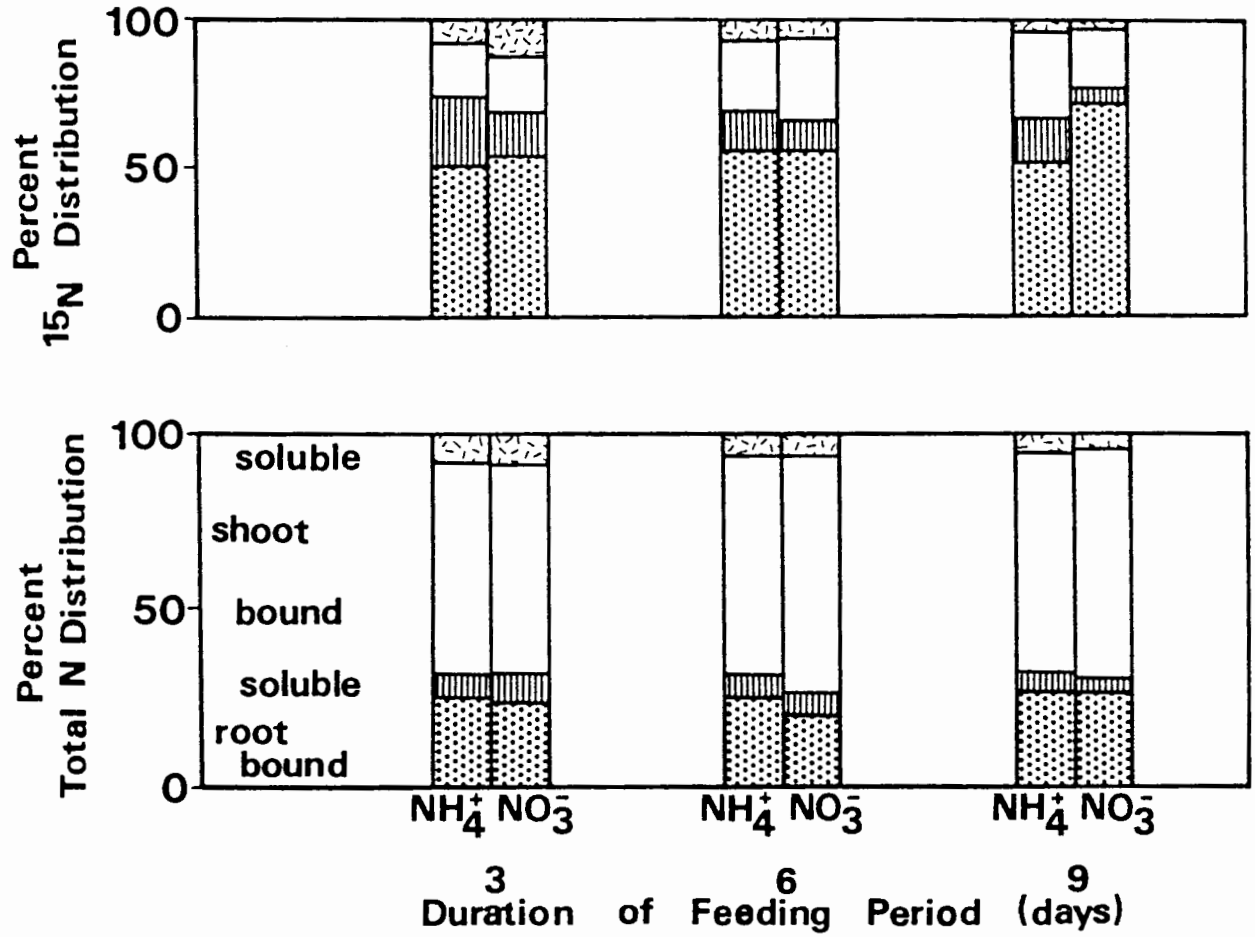

FIGURE 6.1: Total N and ^{15}N distribution in Protea repens root bound , shoot bound , root soluble and shoot soluble fractions after 3, 6 and 9 days feeding with either 2mM $^{15}NO_3$-N or $^{15}NH_4$-N.

The ^{15}N enrichment, size and content of the ethanol-soluble fraction in the roots and shoots (Table 6.2) fluctuated over the 9 day feeding period as ^{15}N was absorbed, assimilated into the soluble fraction and then incorporated into bound N, where the ^{15}N enrichment continued to increase for the duration of the feeding period. No differences in root and shoot ^{15}N distribution patterns between $^{15}NO_3$-N and $^{15}NH_4$-N fed plants were apparent (Fig. 6.1).

DISCUSSION

The capacity of higher plants to absorb and assimilate NO_3-N and NH_4-N varies between species and these differences have been related to growth rates (Lee & Stewart 1978, van de Dijk 1980), soil pH (Havill, Lee & Stewart 1974, Taylor & Havill 1981) and nitrogen form and content of the edaphic environment (Gigon & Rorison 1972, Lee & Stewart 1978).

The results obtained in this study of young plants of *P. repens* indicate a low uptake and assimilation rate of both NO_3-N and NH_4-N (Table 6.1). This is consistent with the results of Lewis and Stock (1978) who showed a limited nitrogen assimilation capacity in another member of the Proteaceae, *Leucadendron xanthoconnus*. Other workers have shown that members of the Australian Proteaceae (e.g. *Banksia serrata*) are not able to

survive feeding regimes with high levels of phosphorus (100 μg ml^{-1} P) and nitrogen (250 μg ml^{-1} N in the ratio of 9 NO_3-N to 1 NH_4-N) whereas B. serrata grown with little or no fertilization with N and P was able to survive and grow (Groves & Keraitis 1976).

Evidence for wild plants belonging to the family Ericaceae, which grow on acid soils, suggest that these plants show a preference for ammonium (Greidanaus, Peterson, Schrader & Dana 1972) and it was at one time suggested that members of the Ericaceae were unable to reduce NO_3-N as the family was thought to lack nitrate reductase (Townsend & Blatt 1966) which has since been disproved by Dirr, Barker and Maynard (1973). Stock and Lewis (1982) demonstrated the presence of nitrate reductase in P. repens and the assimilation of $^{15}NO_3$-N in this experiment provides further evidence (Table 6.1) of the ability of this species to reduce NO_3-N.

The apparent slight preference shown by P. repens in this experiment for NH_4-N uptake and assimilation might be due to ammonium absorption being a passive process which generally occurs at rates greater than those observed for NO_3-N uptake (Higinbotham 1973). In addition, the uptake and assimilation of ammonia under natural conditions of low availability has the lowest assimilatory energetic requirement of any available N form (N_2, NO_3-N or NH_4-N) as no chemical transformation

is required before plant assimilation (Gutschick 1981). The major constraint on NH_4-N uptake is the energetic cost of producing an increased root mass which is required to extract ammonium which moves only short distances within the soil (Gutschick 1981). The energetic constraint in producing root mass is overcome by the seasonal production of proteoid roots in the family Proteaceae (Lamont 1982, 1983) which exploit a large soil volume, particularly pockets of organic matter immediately below the litter layer where rapid ammonification occurs.

Species vary widely in their ability to reduce and assimilate nitrate in the root. Wallace and Pate (1967) and Lewis et al. (1982a) have shown respectively that both cocklebur and barley have low nitrate reductase activity in the roots with the leaves being the principal sites of NO_3-N assimilation, while Grasmanis and Nicholas (1967) have shown that in nitrate-fed apple trees nitrate reduction normally occurs in the fine rootlets. Nitrate reduction in this experiment appears to occur predominantly in the root ^{15}N enrichment and ^{15}N content of the soluble pool and bound fraction of ^{15}N in the shoot are smaller than in the root even after 9 days (Tables 6.2 & 6.3). This is in spite of the findings of Stock and Lewis (1982) who demonstrated nitrate reductase activity to be greater in the leaves

of P. repens than in the roots. In addition Lewis and Stock (1978) demonstrated the occurrence of NO_3-N loading onto the xylem stream of other members of the Proteaceae (P. laurifolia and P. lepidocarpodendron) for later reduction in the leaves of these species. It would appear that P. repens possesses the enzymes responsible for NO_3-N reduction and assimilation in both root and shoot, thus nitrate reduction may occur in both organs although in this experiment NO_3-N reduction appeared to occur mainly in the roots.

The assimilation of nutrient ammonium apparently takes place predominantly in the roots of P. repens as the soluble N fraction of the root is rapidly enriched with ^{15}N and the assimilatory pools have already reached saturation by the third day of ^{15}N feeding. Further, the ^{15}N enrichment of the soluble N fraction in the shoot is low compared with that of the root (Table 6.2) and the allocation of ^{15}N to the bound fraction of the root is much higher than to the bound fraction of the shoot (Fig. 6.1).

As with evergreen chaparral shrub Ceanothus megacarpus Nutt., (Gray & Schlesinger 1983) P. repens shows a minimal response to nitrogen addition and growth of this fynbos plant appears to be similar to other species from infertile habitats which show growth to have little dependence on soil nutrient availability

(Specht & Groves 1966, Rorison 1968) as these plants have the capacity to continue growth using internal reserves after soil nutrients have been depleted.

Protea repens thus appears to have adapted to the conditions prevalent in the later stages of fynbos succession, not by showing an absolute requirement for a particular inorganic N form, but by an ability to absorb and assimilate small quantities of both NO_3-N and NH_4-N to satisfy the low N demand of this slow growing, evergreen species.

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CHAPTER 7

SEASONAL ALLOCATION OF DRY MASS AND NITROGEN IN A FYNBOS ENDEMIC RESTIONACEAE SPECIES *THAMNOCHORTUS PUNCTATUS* PILL..

W.D. STOCK, J.E.M. SOMMERVILLE and O.A.M. LEWIS
Botany Department, University of Cape Town,
Private Bag, Rondebosch, 7700, South Africa.

Strategies which a plant may adopt to overcome nutrient limitation need not include only adaptations to improve nutrient acquisition or reduction of the nutrient demand of the species to one attuned to the low availability of that nutrient, but may include efficient conservation of existing nutrient capital. The quantity of nitrogen mobilized in an endemic member of the Restionaceae was examined to determine the efficiency with which this species is able to withdraw N at organ senescence and to reutilize this nitrogen for growth of other organs. The results of the study are reported in this chapter which has been submitted as a paper for publication to Oecologia. The senior author is responsible for 90% of the work involved in this study.

INTRODUCTION

The Restionaceae are wiry, aphyllous hemicryptophytes which form a dominant structural and floristic element of fynbos vegetation of the South Western Cape Province, South Africa (Taylor 1978, Kruger 1981). The species of this family fill the niche usually occupied by grasses (Poaceae) in neighbouring biomes (Linder 1984). As many as 12 out of 19 of the genera and 290 out of 310 species are endemic to the South Western Cape (Bond & Goldblatt 1984). Limited phenological and hydrological investigations of the growth and water utilization patterns in Restionaceae species in relation to climate have been undertaken (Sommerville 1983, Miller et al. 1983) but investigations of physiological adaptation to the low-nutrient soils has been neglected.

Plant species growing in the low-nutrient soils of the fynbos region must overcome two basic limitations to growth, survival and reproduction. Firstly, they must be able to fix sufficient energy and then reproduce during the short growing periods, as favourable growing temperatures and moisture availability do not coincide in this Mediterranean-climate zone. Secondly, the plants must be able to utilize nutrients efficiently, because the sandstone-derived soils of the area are low in total and available nutrients (Mitchell et al. 1984, Stock & Lewis 1984b).

The life history characteristics of a species, including phenology, growth habit and relative proportion of resources allocated to reproduction, growth and survival, are important

determinants of its success in a given environment (Harper & Ogden 1970, Stearns 1977). Reproduction, growth and survival present conflicting demands on the finite pool of resources available to the plant, and in contrasting habitats different allocation patterns have been shown to be favoured (Gadgil & Solbrig 1972, Abrahamson & Gadgil 1973). Except for phenology, life history characteristics have not been studied in species of the fynbos biome. Phenological studies have to date investigated strategies whereby species from this ecosystem minimize the impact of the Mediterranean-climate on growth and reproduction (Levyns 1964, Bond 1980, Kruger 1981, Sommerville 1983, Pierce & Cowling 1984), but have not addressed the strategies whereby edaphic constraints on these processes are ameliorated.

One such important adaptation to edaphic constraint could be an asynchronous growth pattern. Mooney et al. (1977) has suggested that in perennial, evergreen plants the sequential growth of organs enables species with this characteristic to flourish in low nutrient environments because they have the ability to re-utilize some of the existing plant nutrient capital for development of all organs.

The aim of this investigation was to study aspects of the phenology, growth habit and nitrogen allocation patterns of Thamnochortus punctatus to ascertain if these features could provide an indication of strategies employed by plants to minimize the impact of the Mediterranean-climate and low-nutrient soils on growth, survival and reproduction in the fynbos region of the South Western Cape Province, South Africa.

STUDY AREA

The study was carried out at the Pella Fynbos Biome Intensive Study Site (33°31'; 18°32': area 269 ha) located on the Burgherspost Farm in the Malmesbury district of the Cape Province, South Africa. The vegetation of the Pella site was a mosaic of different ages of coastal fynbos dominated by evergreen sclerophyllous shrubs and Restionaceae. The oldest patches of vegetation were about 20 years old and were dominated by members of the Proteaceae, namely Protea repens L. and P. burchellii Stapf.. The areas of the site at which T. punctatus was sampled (15 month and 5-6 year old plants) were burnt in 1975 and 1980 while a futher area (just outside the Pella site) which was burnt during 1978 was used to sample 3 year old plants. The dominant species found in these post-fire successioal stages were Leucospermum parile L., Phylica cephalantha Sond., Thamnochortus punctatus Pill. and Staberhoa distachya (Rottb) (Brown et al. 1984, Stock & Lewis 1984b). The soil on which the 5-6 year plants were growing was, according to the South African soil classification system, recognized on the basis of soil horizons as the boundary between a Clovelly and a Constantia soil form (MacVicar et al. 1977).The Clovelly soil form is yellow-brown in colour while the Constantia form is white. Both are sandy and of aeolian origin with a depth of 2m and are poor in nitrogen and phosphorus (Mitchell et al. 1984, Stock & Lewis 1984b). The 15 month and 3 year old plants were also sampled on either a Clovelly or a Constantia soil form.

METHODS AND MATERIALS

At 14 week intervals from July 1980-January 1982 five male Thamnochortus punctatus plants (5-6 years old) were selected for dry mass and nutrient distribution studies. Criteria for plant selection were (i) their isolation from other members of the family Restionaceae, as a precaution against confusing the roots of different species and individuals, and (ii) the lack of disturbance of the immediate environment. The positions of sampled plants within the 1 ha sample area were marked so that neighbouring plants, which may have been disturbed by sampling, were not selected at later sampling dates. In January 1982 ten 15 month old plants and five three year old plants were sampled in neighbouring patches of fynbos at Pella which had been burnt during November 1980 and March 1979 respectively.

The plants were harvested by severing the above ground organs of the plant at ground level. The material was divided into the following catagories (Fig. 7.1) on the basis of apparent function and/or age:

1) Developing culms.....Young culms which bore no inflorescences or developing inflorescences up to the anthesis stage.

2) Developing inflorescences..Inflorescences prior to and during anthesis.

3) Mature culms.....Culms which had borne inflorescences in the last flowering season.

4) Matured inflorescences.....Post-anthesis inflorescences.

5) Senescing culms.....Culms which appeared chlorotic.

6) Dead culms.....Completely senesced culms which were brown in colour.

7) Vegetative branches.....Finely divided stem and leaf material borne on the culm or on the rhizome itself. Two age groups were recognized, namely mature and dead vegetative branches.

The below-ground plant parts were sampled by means of a rectangular soil monolith (28cm X 38cm X 10cm) which was centered on the tussock base. The soil monolith containing the root material was then washed through a 2mm sieve. The remaining rhizome and root material was washed futher to remove adhering soil particles and sorted by flotation in water into the following categories:

1) Rhizome.....Dark brown rhizome and culm stumps.

2) Developing roots.....Young roots with a fleshy cortical layer and a white growing tip. In section a white coloured stele and cortical layer were evident.

3) Mature roots.....Roots from which the cortex has been sloughed off exposing a brown endodermal layer. In cross

section a distinct difference between the white stele and light brown endodermis was apparent.

4) Dead roots.....Roots dark brown to black in colour and very brittle. No colour difference between stele and endodermis was apparent.

All samples were dried in a forced draught oven at 80°C for 48 hours before dry mass was determined. Dried T. punctatus material was ground in a Wiley mill to pass through a 40 mesh screen. Material remaining in the grinding chamber was removed and mixed with the finely ground material. This was done to ensure that no segregation of the more resistant material occurred and that the N concentration as determined reflected the total N content of all material constituting that organ. Samples were redried at 80°C for 24 hours and stored in a desiccator. Total nitrogen was determined as follows by a micro-Kjeldahl digestion of triplicate samples of each organ from each of the 5 individuals sampled at each date:

To 0,1000g samples of T. punctatus 3 ml N-free concentrated H_2SO_4 containing 34 g l^{-1} salicylic acid (to include the assay of nitrate and nitrite (Bremner 1965d)), a selenium catalyst tablet (BDH, Poole U.K.) and 0,2 g sodium thiosulphate were added. After digestion in an aluminium block digestor (Nelson & Sommers 1972) the sample was made up to 50 ml with double-deionized water. The ammonium in the digest was determined by an indo-phenol blue procedure described by Allen et al.

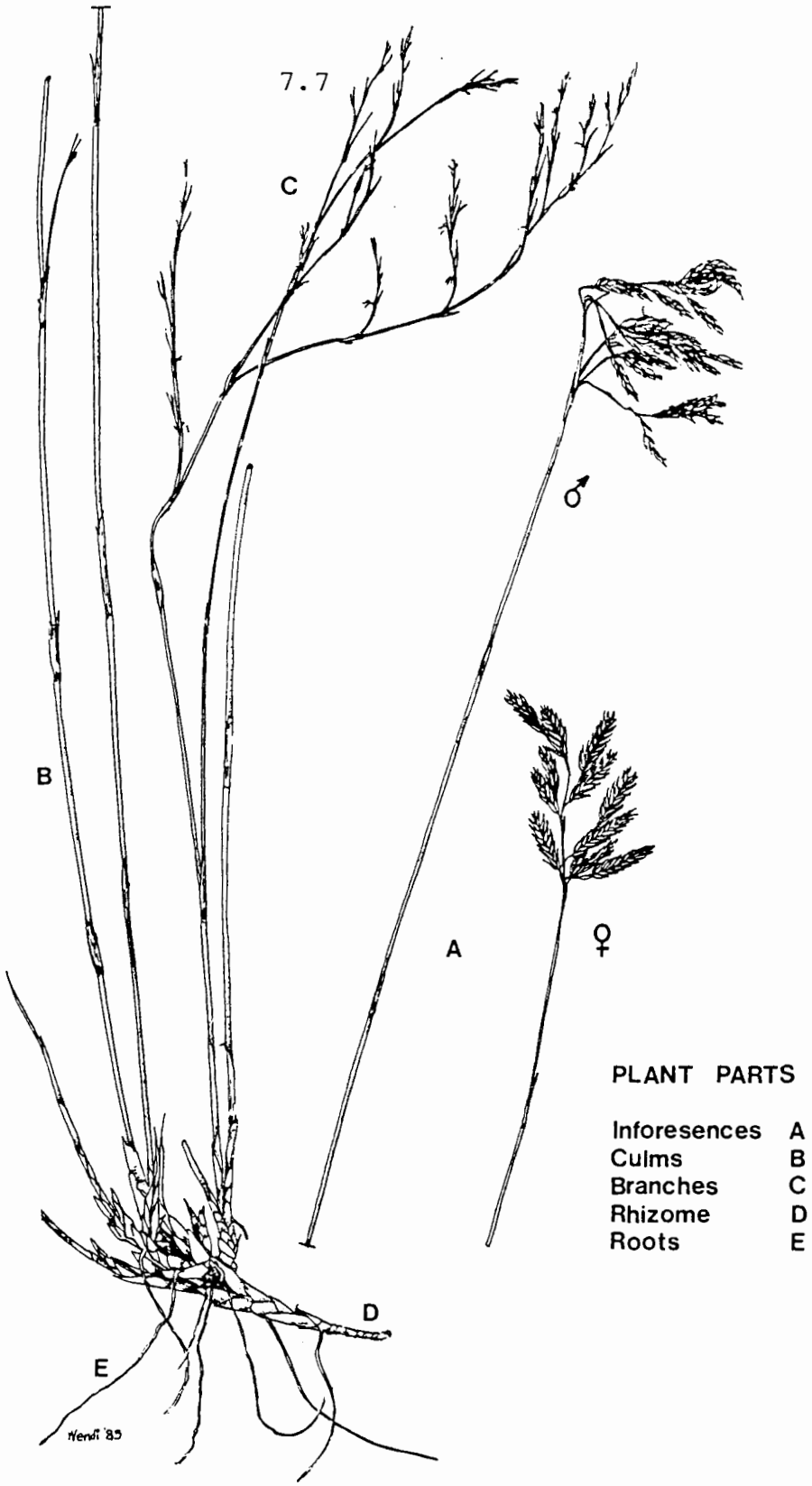

Figure 7.1: The growth form of a five-year old *Thamnochortus punctatus* plant. Plants are dioecious although examples of the structure of both male and female inflorescences are shown.

(1974) modified to include partial neutralization of the acid digest with NaOH before colour development. This was done in order to eliminate any possible interference in colour development due to the very low pH of the digests. If the coefficient of variation (CV%) between replicates of the same sample was above 3% for above-ground organs and 5% for root or rhizome material, futher replicate determinations were performed to obtain the required degree of variance.

RESULTS

Changes in dry mass allocation patterns with plant age.

The climate during the study period showed characteristic features of a mediterranean climate with winter rainfall and high summer temperatures (Fig. 7.2). No seedlings were evident until 15 months after an accidental fire which occurred during spring (November 1980). The juvenile stage connsisted of vegetative branches, rhizome and roots without culms of reproductive material (Fig. 7.3). The 3 year old plant produced culms and inflorescences but the vegetative branches still constituted 30% of the above ground plus rhizome total (Fig. 7.3). As plants aged, vegetative branches decreased in importance until in the 6 year old plant they contributed only 1,2% of the dry mass (Fig. 7.4).

The dry mass allocation of dead material increased with the age of the plants, reaching a total allocation of up to 21,6% in the 6 year old plants (Fig. 7.4). Culms of *T. punctatus* appeared to senesce after 15-18 months and were completely dead after

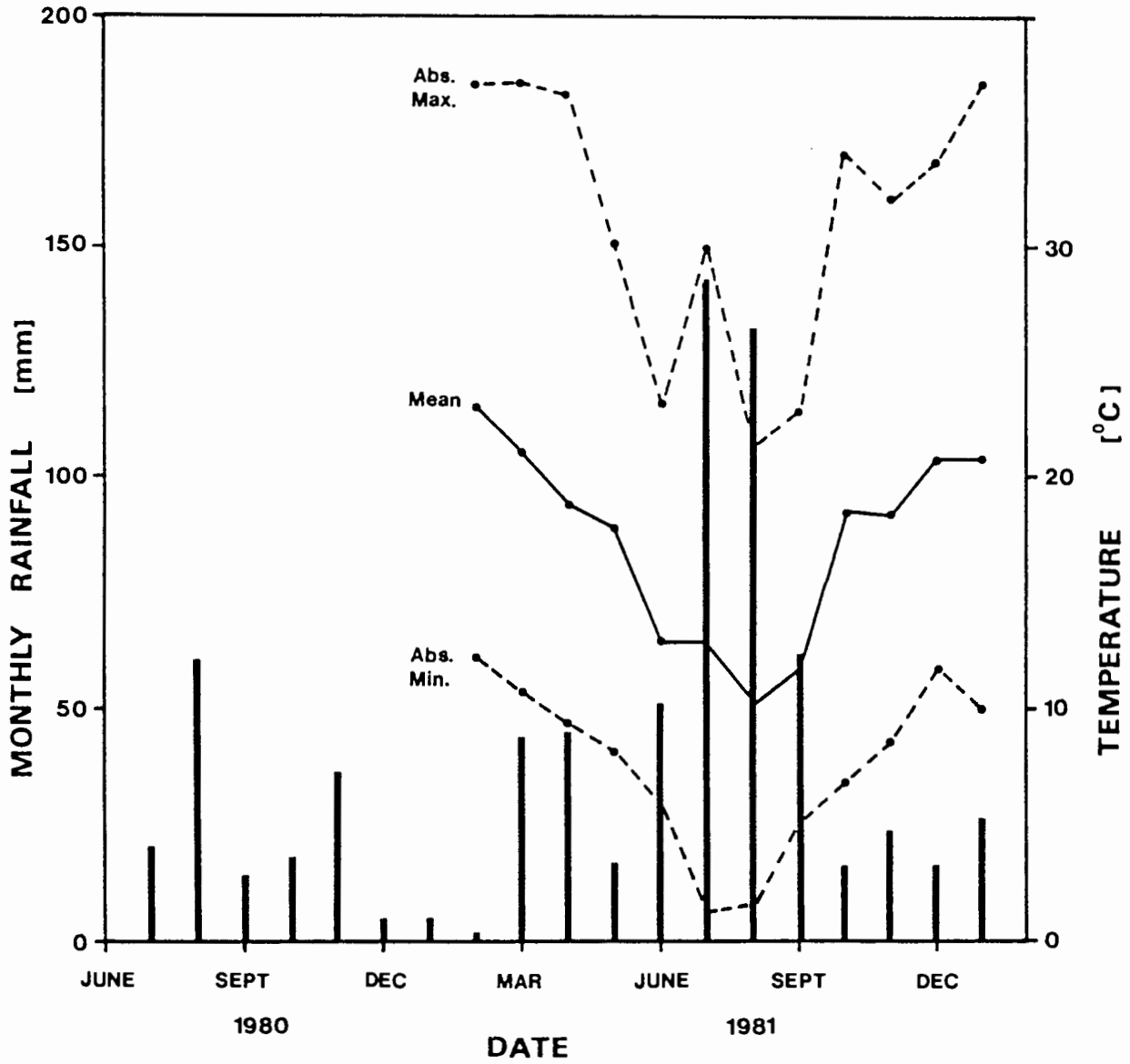

Figure 7.2: Rainfall and temperature data collected at the Pella weather station during the experimental period from July 1980-January 1982. Temperature data was only collected from February 1981-January 1982.

32-34 months.

Seasonal production and allocation of dry mass.

Seasonal production and allocation of dry mass determined in 5 to 6 year old plants over an 18 month period from August 1980 until January 1982 showed consistantly significant ($p < 0,001$) seasonal changes in dry mass increase and partitioning for both growth periods studied (Fig. 7.4). On a mean plant basis, T. punctatus showed the greatest mass increase of above-ground organs in spring to summer (Fig. 7.4: October-January for 1980-81 and 1981-82). The development of the culms followed a definite seasonal sequence of mass increase; they elongated rapidly in spring until, in summer, they reached a maximum of 50,8% of above ground plus rhizome dry mass (February 1981, Fig. 7.4). During autumn and winter the developing culms matured and at the beginning of spring costituted 45,1% of plant dry mass. Senescence of culm material was not a definite seasonal event. Between February and May 1981 culms appeared to have senesced while the second cohort appeared to have senesced between September 1981 and January 1982. The dead culm standing dry mass increased during the summer months of both years studied, the second year showing a particularly large increase (Fig. 7.4).

Seasonal rhizome growth appeared to be more variable between the years than culm development. Dry mass of this organ increased during spring and summer of 1980-81 while in 1981-82 dry mass increase was greatest during the rainfall period of winter to spring (July - September 1981, Fig. 7.4).

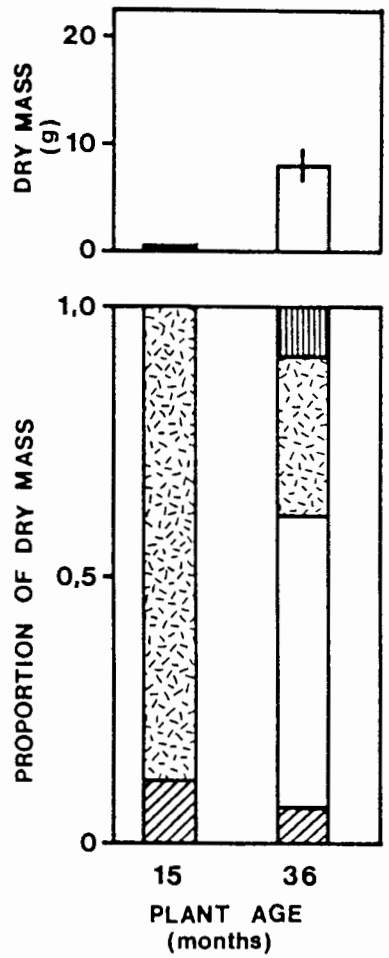

Figure 7.3: The dry mass and proportion of dry mass of above-ground plus rhizome plant parts allocated to rhizome ▨, culm □, branch ⁝⁝ and inflorescence ▥ organs in 15 month old and 3 year old *T. punctatus* plants.

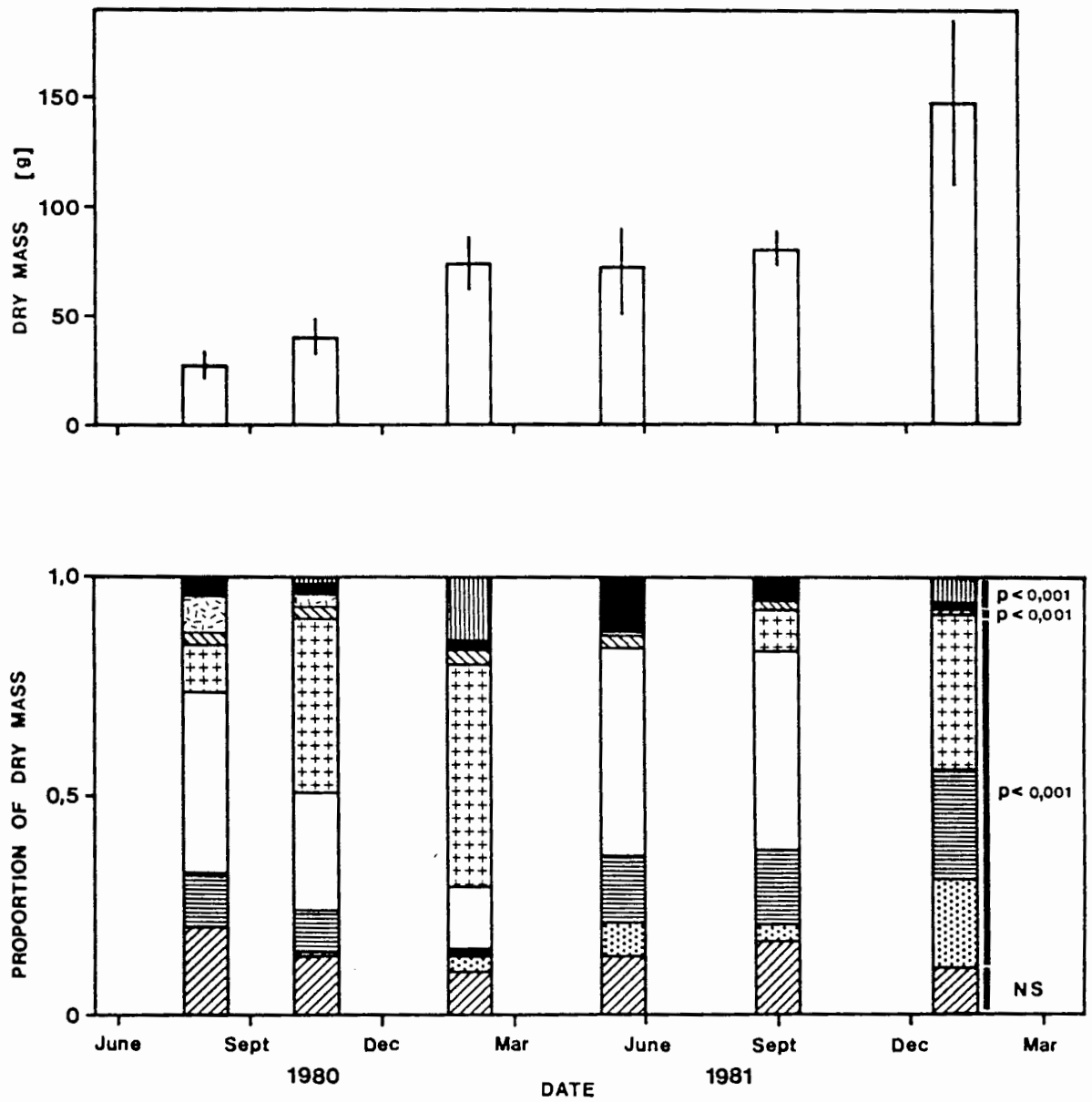

Figure 7.4: The dry mass and proportion of dry mass of above-ground plus rhizome plant parts allocated to rhizome ▨, developing culm ⊞, mature culm □, senescing culms ▤, dead culms ⁘, developing inflorescences ▥, mature inflorescences ■, mature vegetative branches ⁘ and dead vegetative branches ▧ in 5-6 year old male *T. punctatus* plants. Seasonal significance of allocation patterns is shown (ANOVA of arcsin transformed proportion data).

Inflorescence buds were first found on the developing culms during late spring and their dry mass increased until late summer (Fig. 7.4). Reproductive effort (relative allocation of mass to reproductive organs) reached a maximum of 14,5% of above ground plus rhizome dry mass in February 1981. After anthesis occurred during autumn matured inflorescence bracts were shed; they costituted less than 1% of above ground plus rhizome dry mass in the following flowering year (February 1981- January 1982, Fig. 7.4).

Although the roots of this species are short in length and very shallow the root sampling technique involved a possible loss of root material extending beyond the limits of the monolith. Hence no conclusions concerning total root mass could be made. Nevertheless the seasonality of the root material in the monolith showed that developing roots were found in the rainfall months of winter and spring (Fig. 7.5).

Nitrogen uptake, season and quantity.

The dry mass of the rhizome plus above ground organs peaked during the summer months upon completion of culm development and the total N content of these organs closely followed this pattern (February 1981 & January 1982, Fig. 7.6). From May 1981 to culm maturation in January 1982, 58,5% of the nitrogen contained in these organs was extra-shoot nitrogen, that is it came from the roots either by direct uptake or mobilization of existing root nitrogen. The major portion (35,1%) of this exogenous N was taken up or remobilized during spring and summer (September 1981-January 1982). This quantity is similar to the 37,7% of

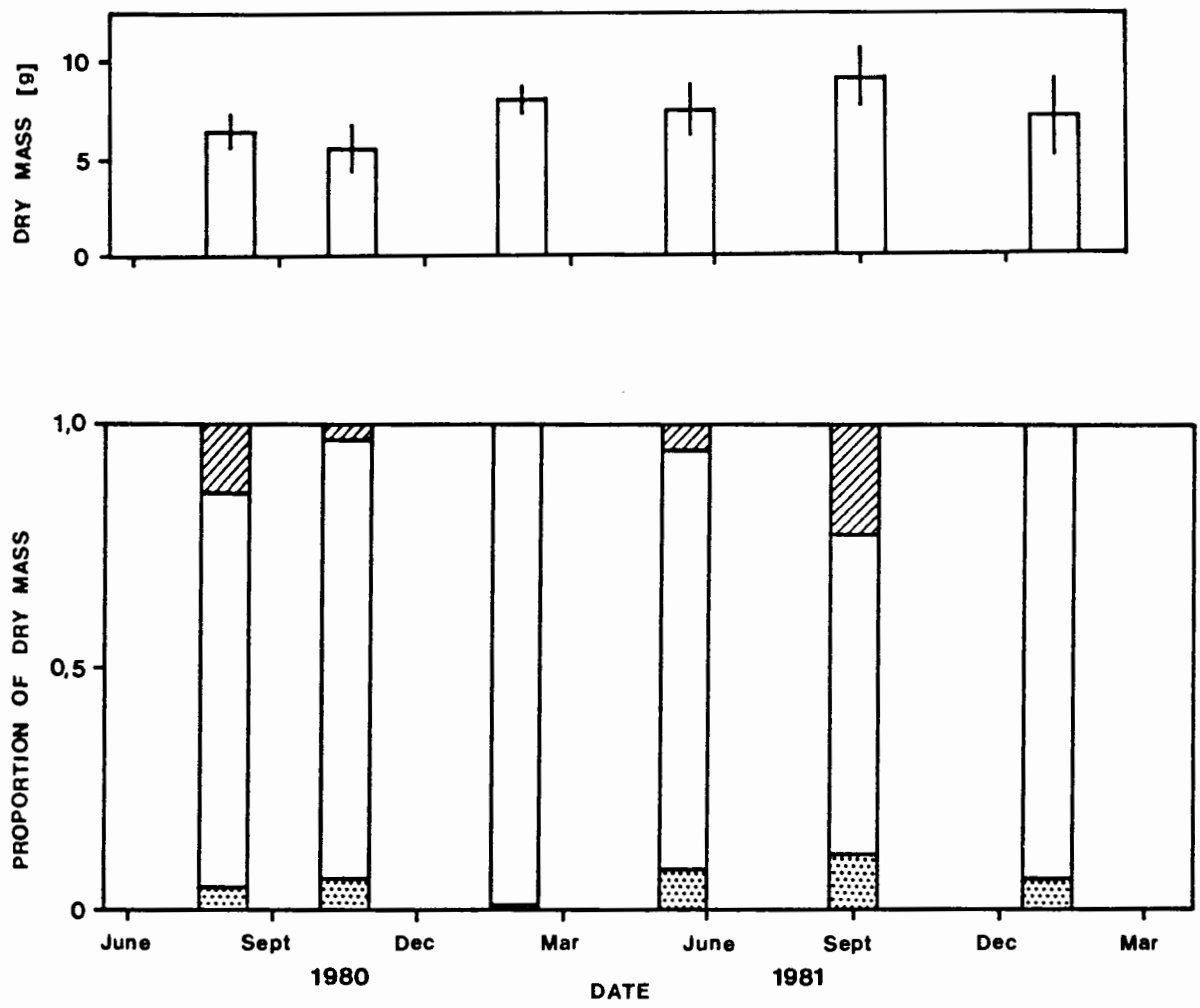

Figure 7.5: The root dry mass and the proportion of dry mass allocated to developing root ▨, mature root □ and dead root ⁘ categories. Roots collected from 38 x 28 x 10 cm soil monoliths beneath 5-6 year old male *T. punctatus* plants.

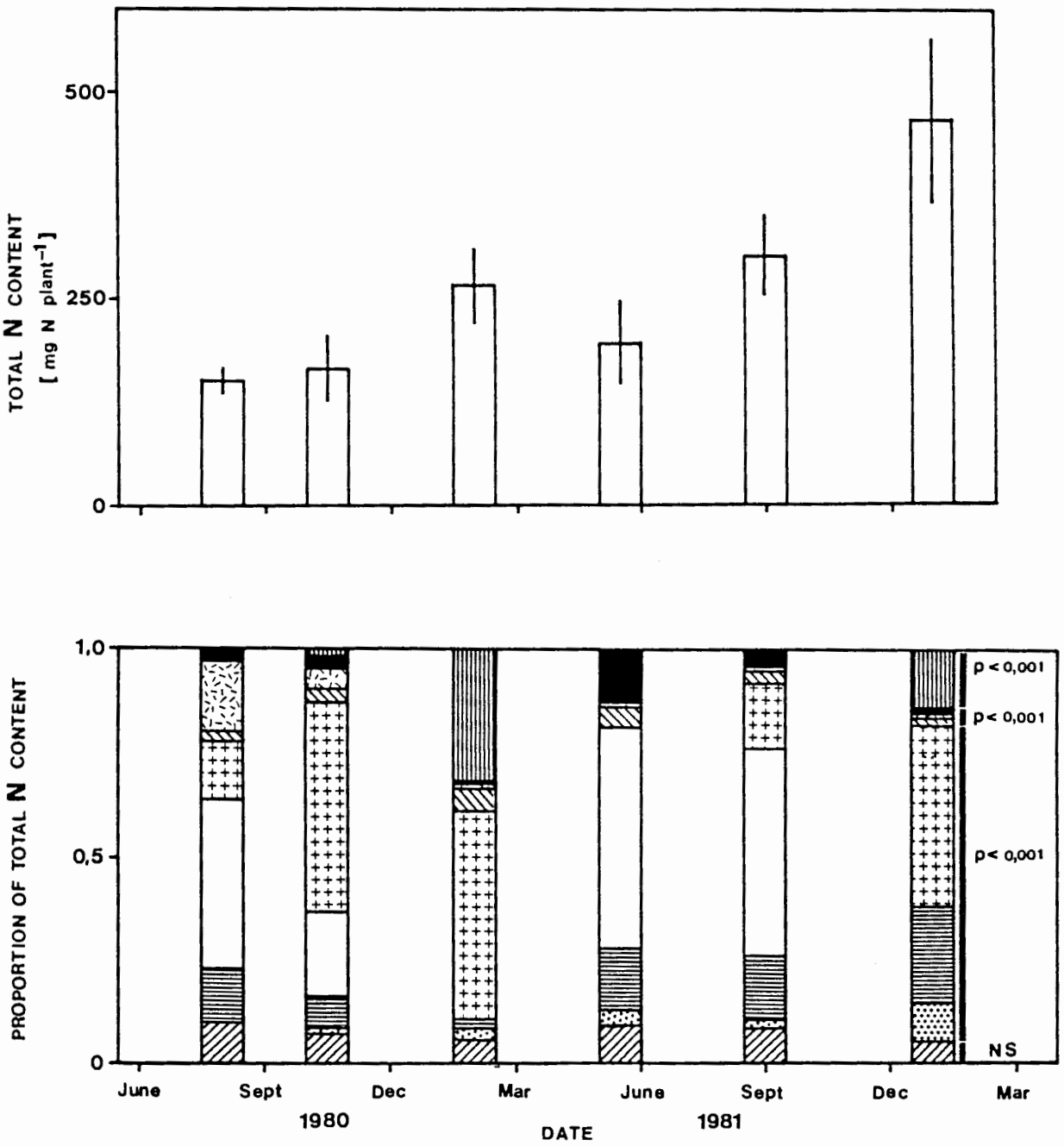

Figure 7.6: The total N content and proportion of total N of above-ground plus rhizome plant parts allocated to rhizome ▨, developing culm ⊞, mature culm □, senescing culm ▤, dead culm ⊡, developing inflorescences ▥, mature inflorescences ■, mature vegetative branches ⊠ and dead vegetative branches ▧ in 5-6 year old male T. punctatus plants. Seasonal significance of allocation patterns is shown (ANOVA of arcsin transformed proportion data).

exogenous N incoporated in the previous spring to summer season. Changes in root dry mass and total N content within the limits of the soil monolith showed no consistent seasonal pattern although a marked decrease in root total N was found from September 1981 to January 1982 (Fig. 7.7) which coincided with the increased N content of the above ground organs. The major loss of N from the above ground plus rhizome organs occurred during autumn when the inflorescences matured and pollen was shed (Fig. 7.6; eg. 26% of total N present in February 1981 was lost before May 1981). Dead culms and branches remained attached to the plant throughout the study period and were therefore not considered as sources of N loss.

Seasonal concentration and allocation of plant nitrogen.

A study of the distribution of N within the rhizome plus above ground organs of T. punctatus during the period of study revealed seasonal changes in total N allocation patterns between organs. Each change was related to one of the phenological phases of the plant. The spring to summer periods from September to January were characterized by a substantial allocation of N to developing culms (Fig. 7.6). Upon the completion of the culm growth phase, inflorescences were borne terminally on the culms, with 32% of the plant's rhizome plus above ground N being allocated to reproductive structures (Fig. 7.6; $p < 0,001$). The decreased importance of branches at this and later stages in the life history of the plant was evident by the reduced amount of N allocated to these structures (a decrease of

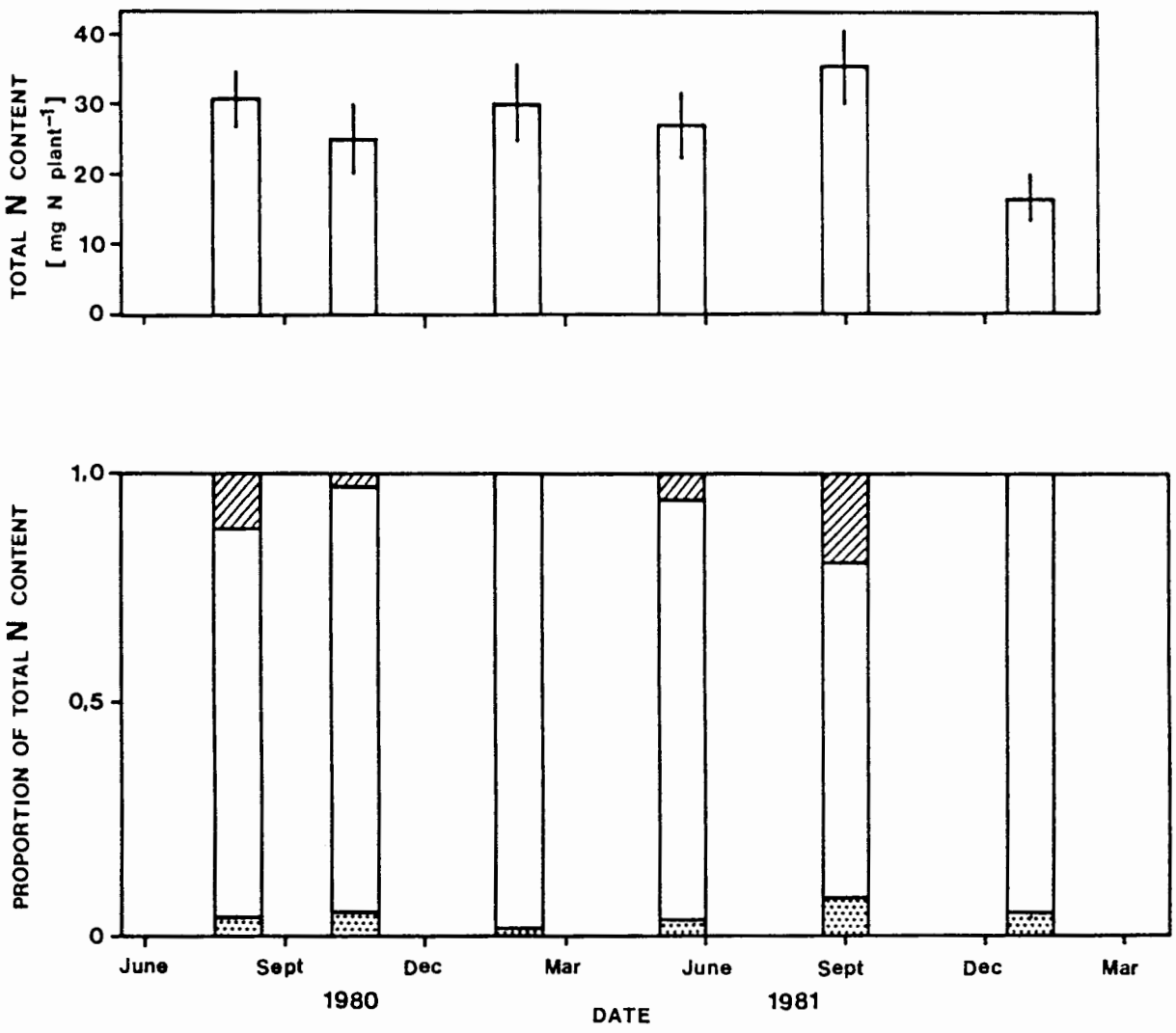

Figure 7.7: The root total N content and the proportion of total N allocated to developing root ▨, mature root □ and dead root ⁘ catgories. Roots collected from 38 x 28 x 10 cm soil monoliths beneath 5-6 year old male T. punctatus plants.

19% to 3% of total rhizome plus above ground total N was recorded). Nitrogen allocation to rhizome production and maintenance remained relatively constant with small, non-significant variations.

The total N concentrations of the organs differentiated at each sampling date showed that the young developing tissues had the highest N concentrations of each organ (Table 7.1). Seasonal fluctations in total N concentration were evident in all the culm categories with apparent storage of N occurring during winter (May-September 1981, Table 7.1) in the mature culms when root growth was highest. In spring and summer, N concentrations in the culms decreased while increased total N in developing culms was found. This was followed by the increased production of inflorescences and the channelling of N to these structures (Table 7.1). The difference between the N concentration of the mature culm stage in August 1980 and the standing dead culms in January 1982 (the same cohort aged to the next category) showed that 70% of the N present at the mature stage had been remobilized and withdrawn or lost due to leaching as the culms senesced. The branch material showed a 35% withdrawal or loss of N over the same period. Back translocation of N from senescing above ground material contributed 14% of the total N present in the rhizome plus above ground organs during January 1982 while 52% was exogenous N and 24% was still retained in senescing and standing dead material.

TABLE 7.1: Seasonal total N concentrations (mg g^{-1} dry mass) of the different organs of *T. punctatus* plant material collected at the Pella site.

PLANT PART	DATE					
	AUGUST 1980	OCTOBER 1980	FEBRUARY 1981	MAY 1981	SEPTEMBER 1981	JANUARY 1981
INFLORESCENCE						
Developing	-	4,56±0,36	8,12±0,53	-	-	8,00±0,43
Mature	4,49±0,23	4,80±0,53	2,87±0,25	3,03±0,27	3,84±0,35	4,04±0,41
BRANCH						
Mature	10,21±0,77	5,89±0,59	5,44±0,27	6,73*	7,38±1,00	6,88±0,64
Dead	4,74±0,39	3,81±0,39	4,21±0,17	4,58±0,17	4,67±0,27	5,65±0,45
CULM						
Developing	6,51±0,48	4,97±0,26	2,97±0,22	-	5,48±0,37	3,66±0,28
Mature	7,02±1,16	3,30±0,17	2,44±0,25	3,41±0,30	4,48±0,52	-
Senescing	6,31±0,87	3,16±0,31	3,18±0,22	2,81±0,33	3,86±0,55	2,86±0,16
Dead	-	2,56±0,25	2,30±0,18	1,80±0,17	2,58±0,31	2,09±0,24
RHIZOME	3,16±0,61	2,14±0,34	1,88±0,20	2,08±0,17	1,71±0,21	1,76±0,15
ROOT						
Developing	4,27±0,27	4,00±0,45	-	3,92*	3,13±0,29	-
Mature	5,13±0,33	4,82±0,35	3,50±0,49	3,88±0,42	3,84±0,10	2,45±0,22
Dead	3,50±0,15	3,06±0,35	2,62±0,22	2,40±0,27	2,52±0,21	2,31±0,37

MEAN ± SEM (n=5 except* n=2)

DISCUSSION

The developing stages in the life-history of T. punctatus after fire disturbance appears to be characterised by two distinguishable phases. The first or establishment phase occurred upon the germination of T. punctatus in the second year after fire. This phase consisted of the production of vegetative branches which formed 100% of the above ground dry mass. Vegetative branches with their higher surface area and total N concentrations would to increase the carbon acquisition of the plant by increasing photosynthetic area while nutrients and water were more available in the burnt over soils (Stock & Lewis 1984b). The second phase of development continued throughout the lifespan of the plant and involved the production of reproductive material. The reproductive phase was easily recognized by the decreased importance of vegetative branches and the increased production of inflorescence bearing photosynthetic culms. The first inflorescences recorded on T. punctatus were found on 3 year old individuals.

The annual growth rhythms of many fynbos and South Australian plants show rapid vegetative growth during summer, the dry period of the year. According to Levyns (1964), Groves (1965) and Specht (1975) this suggests that these plants had their origin under different climatic circumstances than the mediterranean-climate experienced at present. Thamnochortus punctutas appears to reflect a similar anomalous growth pattern with maximum culm elongation in spring to early summer (Sommerville 1983) while greatest dry mass increase follows this

in the middle of summer (Fig. 7.4). However, our study showed that the growth phases of the various organs of T. punctatus were sequential and in phase with the seasonal changes of environmental factors. The roots and rhizome appeared to develop when soil moisture was highest; vegetative culm growth occurred in spring and summer when temperatures increased while reproduction occurred in late summer to autumn when, as Sommerville (1983) and Miller et al. (1983) reported water stress was greatest.

The low-nutrient soils of the South Western Cape Province may give T. punctutus with its asynchronous growth pattern a competitive advantage over plants with synchronous growth habits. This is because the asynchronous habit allows a portion of the nutrient capital to be recycled between different organs during each of their growth periods. Mooney et al. (1977) argued that this time separation is in part due to a limitation of building materials (carbon and nutrients) and that priority is given at any one time to the critical growth phase.

During the rainfall period characteristic of this mediterranean-climate zone from early winter to spring 23% of the annual uptake or retranslocation of total N from root to above-ground organs occurred, while during spring and summer a futher increase of 35% was present in the above-ground plus rhizome organs. All N taken up was directed to the above-ground organs, in particular the mature culms, and no evidence of large stores of N in the root and rhizome were apparent (Table 7.1). In spring to early summer the N taken up was used directly for culm

development. Mooney and Rundel (1979) have shown that chamise (Adenostoma fasiculatum H.&A.), a chaparral dominant, takes up N and P during the winter rainy season prior to any above ground growth. It was shown that these minerals were stored in the old evergreen leaf tissue before being translocated to new growth in spring. The increase in mature culm N concentration and proportion of total N allocated to culms during winter suggests that T. punctatus employs a similar strategy and that the evergreen culms provide a sink for N during the N uptake but above-ground growth-dormant periods.

The correlation of evergreenness with nutrient poor substrates has frequently been recorded (Monk 1966, Beadle 1966, Small 1972) and not only is leaf longevity important to the period where minerals may be used before they are lost, but the quantity of the elements reabsorbed at senescence is critical. The efficient retranslocation of nutrients has been suggested to increase nutrient use efficiency (Rundel 1982). Our data showed that up to 70% of total N present in mature culms may be withdrawn before death occurs. This appears to be similar to Scots pine (Pinus silvestris L.) colonizing low nutrient soils which retranslocated 76% of N from needles at senescence whereas a species from a more mesotrophic site only retranslocated 7% of total N (Alnus glutinosa (L)) before leaf fall (Starchurski & Zimka 1981). Retranslocation of N in different species of mediterranean-type climate zones showed a range of N withdrawl and retranslocation values of between 17-57% and 22-56% in California and Chile respectively. The highest efficiency

recorded in these systems was well below the 72% found in Ledum groenlandicum in a Canadian bog (Small 1972) and 63% found in Lycopodium annotium, a subartic species growing in soils poor in nutrients (Callaghan 1980).

Harper and Ogden (1970) suggested that energy allocation may be less important in the evolution of plant strategies than allocation of a scarce resource such as nitrogen and Bell et al. (1979) found no obvious adaptive significance to species specific patterns of biomass allocation in Mojave Desert winter annuals. However subsequent examination of nitrogen allocation patterns of the species revealed a variety of mechanisms for growth in N-poor desert soil and that the N poor species appear to allocate N to reproduction at the expense of vegetative organs throughout the life history of the plant (Williams & Bell 1981). The reproductive allocation of dry mass in male plants of the dioecious T. punctatus was 14,4% whereas reproductive N allocation was 32% and this occurred at the expense of the vegetative organs, in particular the mature culms (Fig. 7.6). The differences between biomass allocation and N allocation patterns described showed, as suggested by Abrahamson and Caswell (1982), that plant strategy analysis in low nutrient environments should include nutrient allocation studies as biomass allocation cannot be assumed to reflect strategies evolved to overcome nutrient limitation.

Thus Thamnochortus punctatus plants represent an efficient and specialized system capable of maintaining themselves in the S.W. Cape on low nutrient soils under a

mediterranean-type climate by their possession of an evergreen, perennial growth habit in which an asynchronous organ growth pattern enables the plant to absorb, conserve and re-cycle scarce nutrients within its almost closed system of interconnected organs.

ACKNOWLEDGEMENTS

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CHAPTER 8

ATMOSPHERIC INPUT OF NITROGEN TO A COASTAL FYNBOS ECOSYSTEM OF THE SOUTH WESTERN CAPE PROVINCE, SOUTH AFRICA.

W.D. STOCK and O.A.M. LEWIS
Botany Department, University of Cape Town,
Private Bag Rondebosch, 7700, South Africa.

In a low N status ecosystem, such as coastal fynbos, the losses of nitrogen due to volatilization by recurrent fires is an important nutritional determinant of future fynbos survival. The input of N in bulk precipitation to a coastal fynbos ecosystem was studied over two years in order to determine whether this is a siginificant source of replacement N for that lost by regular burning of this vegetation type. The results are presented in this chapter which has been submitted as a paper for publication to the South African Journal of Botany.

INTRODUCTION

The nitrogen composition and content of atmospheric precipitation has received much attention in the past, and it is well known that combined nitrogen in precipitation consists of ammonia, nitrate, nitrite and organically bound nitrogen (Eriksson 1952, Jones 1960, Gore 1968). Hutchinson (1944) proposed that the combined N in precipitation originates from

(a) the soil and the ocean,

(b) from fixation of atmospheric nitrogen (electrical, photochemical and in meteorite trails) and

(c) from industrial contamination.

The quantity of nitrogen brought into the soil via atmospheric deposition is normally too small to be of significance in crop production. This nitrogen may nevertheless represent an important income to the nitrogen economy of undisturbed natural ecosystems on nutrient poor soils (Carlisle *et al.* 1966, Allen *et al.* 1968, Christensen 1973). Such an ecosystem is that found in the coastal fynbos of the winter rainfall region of the South-Western Cape Province, South Africa, which occurs on acidic, sandy soils of low nutrient status. Nitrogen is in particularly low concentrations in this ecosystem where total N values range from 250 to 350 kg N ha^{-1} at the soil surface (0 - 10 cm) (Stock &

Lewis 1984b).

This paper describes the input of combined nitrogen from bulk precipitation to a coastal fynbos ecosystem at Pella, near Cape Town. It further describes an investigation of the local recycling of dust aerosols carried out by comparing the input of inorganic N to collectors situated at 1,5 m and 4 m above the soil surface.

STUDY AREA

The study of atmospheric deposition of nitrogen in bulk precipitation was carried out at the Pella Fynbos Biome Intensive Study site (33^{o} 31' S : 18^{o} 32'E : area 269 ha) located at the Burgherspost Farm in the Malmesbury district of the Cape Province, South Africa. The vegetation of this mediterranean climate zone is coastal fynbos dominated by evergreen sclerophyllous shrubs and Restionaceae. The oldest patches of vegetation are about 20 years old and are dominated by two members of the Proteaceae, Protea repens L. and P. burchellii Stapf. Other areas of the site were burnt in 1975 and 1980; the major species found in these locations are Leucospermum parile L., Phylica cephalantha Sond., Thamnochortus punctatus Pill. and Staberhoa distachya (Rottb.) (Brown et al. 1984). The average height of the vegetation canopy was

approximately 1 m. The soil is yellow-brown, well drained, sandy and of aeolian origin with a depth of 2 m and a low nitrogen and phosphorus content (Mitchell et al. 1984, Stock & Lewis 1984b).

MATERIALS AND METHODS

Bulk precipitation samples (wet fall and dry fall) were collected for analysis at weekly intervals from the Pella research site for the period June 1980 until June 1982. Precipitation was collected in 5 polythene funnels 16,8 cm in diameter, each connected to a 2,5 l collecting bottle. The funnels were surrounded with metal spikes at 1 cm intervals to prevent birds from perching, and were covered with a 1 mm^2 fibre glass mesh to prevent contamination from insect and plant debris. The funnel was positioned 1,5 m above soil level; plant canopy height in the study area was 1 m. The funnels and bottles were thoroughly acid washed and rinsed with chloroform and double deionized water before being placed in the field. To each collecting vessel 0,5 ml of a preservative solution was added (500µg ml^{-1} mercuric chloride dissolved in 1M HCl). The collecting vessels were sampled at weekly intervals and 50 ml aliquots were removed for analysis. If no rainfall occurred, 30 ml of double deionized water was added to rinse the funnel and collecting bottle. This solution was then analysed for

the soluble and total input from dryfall. The funnel and collecting vessel were washed with chloroform and two rinses of double deionized water after sample collection before they were returned to the field. Samples showing obvious signs of contamination by bird excrement or insects were discarded and the collecting equipment cleaned and replaced in the field. From June 1981 a further five collecting vessels were placed in the field 4 m above ground level. Sampling of these vessels occurred at weekly intervals in the same manner described for the 1,5 m collectors. All samples collected were stored at $4^{o}C$ for 4 to 6 weeks until analysis.

Nitrate was determined by the Szechrome NAS analytical reagent method (R & D Authority, Ben Gurion University of the Negev Applied Research Institute, Israel). To 0,5 ml of sample, 2,5 ml of Szechrome NAS reagent were added (prepared by adding 5 g of NAS reagent powder to 1 l of mixed equal volumes of concentrated phosphoric acid and sulphuric acid). The reaction mixture was agitated and colour development was read at 570 nm after 5 min. Nitrate samples were prepared in double deionized water in the range 0.2µg ml^{-1} to 1,5µg N ml^{-1}. Ammonium in precipitation was determined on a 3 ml sample volume by an indo-phenol blue procedure modified from Allen et al. (1974) as

described by Stock (1983). Ammonium standards were prepared in the range 0,1µg N ml^{-1} to 1,5µg N ml^{-1}. Nitrite was determined on the samples taken during the first 3 months of the study but its estimation was discontinued thereafter as NO_2-N was found to be present in only trace amounts.

Total N precipitation was determined every 3 to 4 months (in the first year) on weekly samples bulked together in proportion to the rainfall volume. In weeks where less than 1 mm or no rainfall occurred 1 ml of collector rinse water was added. The bulked sample was divided into three aliquots and to each aliquot, 3 ml of concentrated (NH_4-N free) AR H_2SO_4 was added. Approximately 0,5 g of Devarda's alloy was added to each of the 3 replicates to reduce NO_3-N to NH_4-N and the samples were each evaporated down to 5 ml. This concentrate was then decanted into a Kjeldahl digestion tube and a selenium catalyst tablet containing K_2SO_4 (BDH, Poole, UK) was added. After the remaining water had been driven off a Kjeldahl digestion was carried out. Ammonium in the digest was determined by collection in 0,02M HCl after alkalisation of the digest with 50% (w/v)NaOH.

RESULTS

The annual rainfall of the 1980-81 and 1981-82 years was 381 mm and 466 mm respectively (Table 8.1) and occurred predominately in the winter months of June to August of both years studied (37,1% and 45,2% for 1980-81 and 1981-82 respectively) (Fig. 8.1). Total nitrogen input was determined only for the 1980-81 year and amounted to 1,99 kg ha^{-1} (Table 8.1). Inorganic nitrogen, as NO_3-N and NH_4-N, accounted for 56% of the input for this period (1,12 kg ha^{-1}) and the quotient of nitrate to ammonium was approximately 1. Inorganic nitrogen input in the 1981-82 year was 1,79 kg ha^{-1} also with an ammonium to nitrate quotient of 1 (Table 8.1). The seasonal distribution of nitrate and ammonium input differed between the years studied (Figs. 8.2 & 8.3). In the 1980-81 year greatest inputs of NO_3-N and NH_4-N were recorded in winter (33,4% and 40,4% respectively). The distribution of NO_3-N input for the remainder of the year was 31,3%, 20,6% and 14,7% for autumn, summer and spring respectively (Fig. 8.2). Ammonium input for the 1980-81 year followed the same seasonal pattern as nitrate, with 21,6%, 20,5% and 17,5% for autumn, summer and spring respectively (Fig. 8.3). Nitrate input during the 1981-82 year was highest in autumn (42,1%) and lowest in winter (15,9%). Spring and summer NO_3-N input values were 25,7% and 16,3% respectively. Ammonium input

TABLE 8.1: Annual rainfall and bulk precipitation inputs of organic N, NO_3-N and NH_4-N to a coastal fynbos ecosystem at Pella, South Africa over a two year period.

	YEAR	
	June '80 - May '81	June '81 - May '82
Annual rainfall (mm)	381	466
Organic nitrogen (kg N ha^{-1} y^{-1})	0,87	-
Nitrate (kg N ha^{-1} y^{-1})	0,58	0,95
Ammonium (kg N ha^{-1} y^{-1})	0,54	0,84

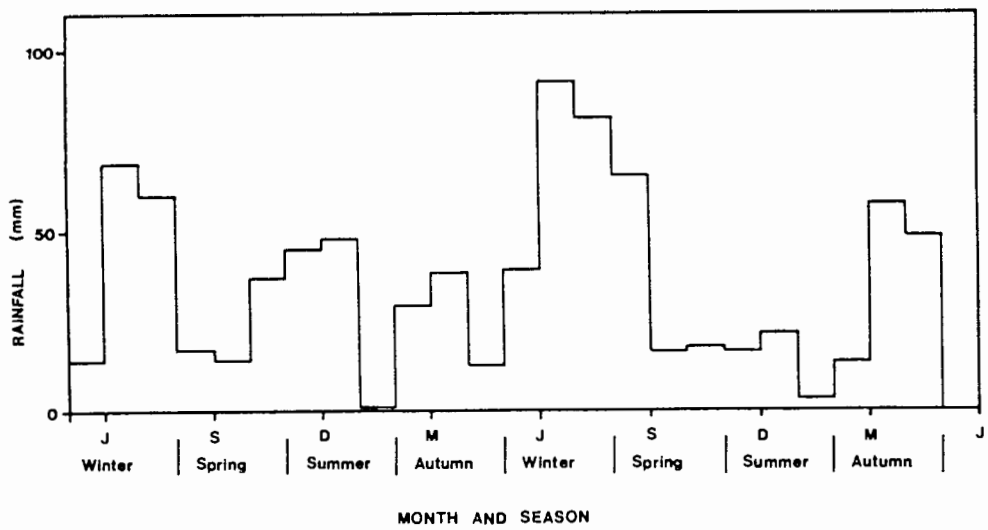

FIGURE 8.1: Monthly rainfall at Pella, South Africa during the study period of 1980-81 and 1981-82.

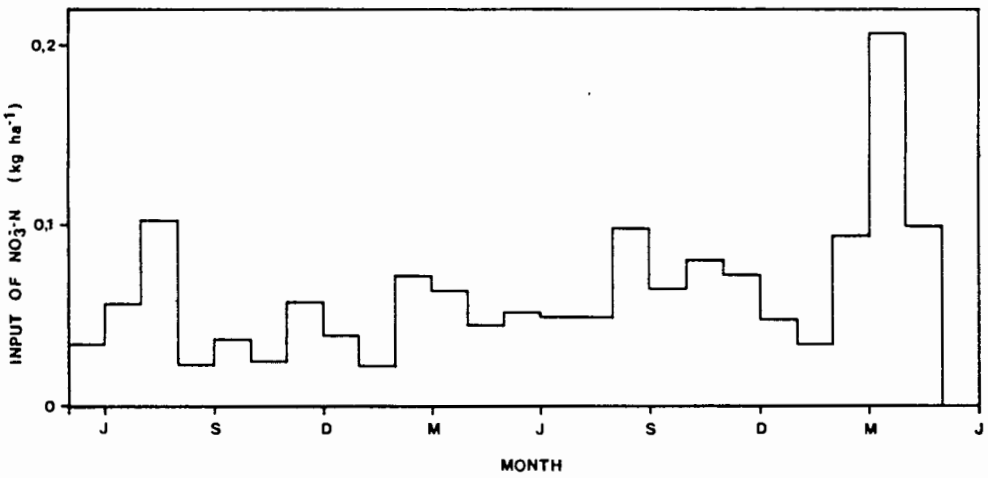

FIGURE 8.2: Monthly input of nitrate in bulk precipitation at Pella, South Africa during the study period of 1980-81 and 1981-82.

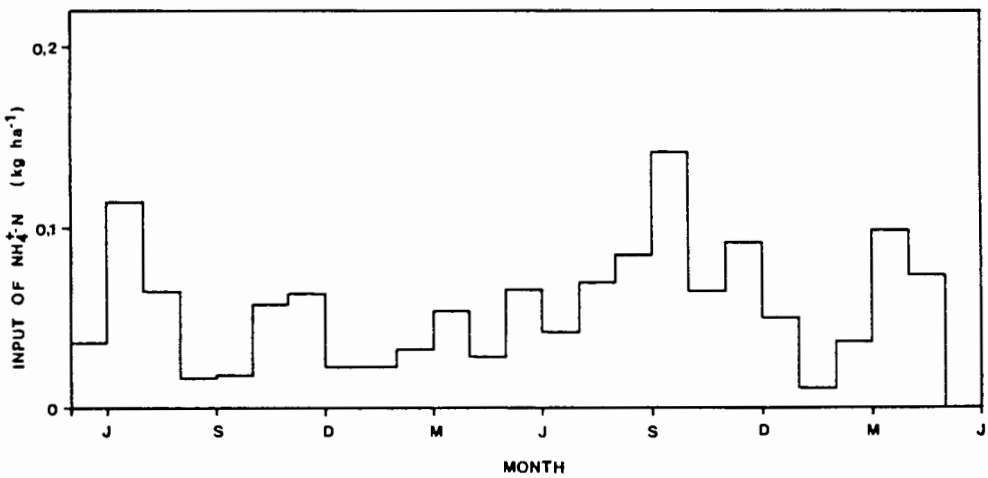

FIGURE 8.3: Monthly input of ammonium in bulk precipitation at Pella, South Africa during the study period 1980-81 and 1981-82.

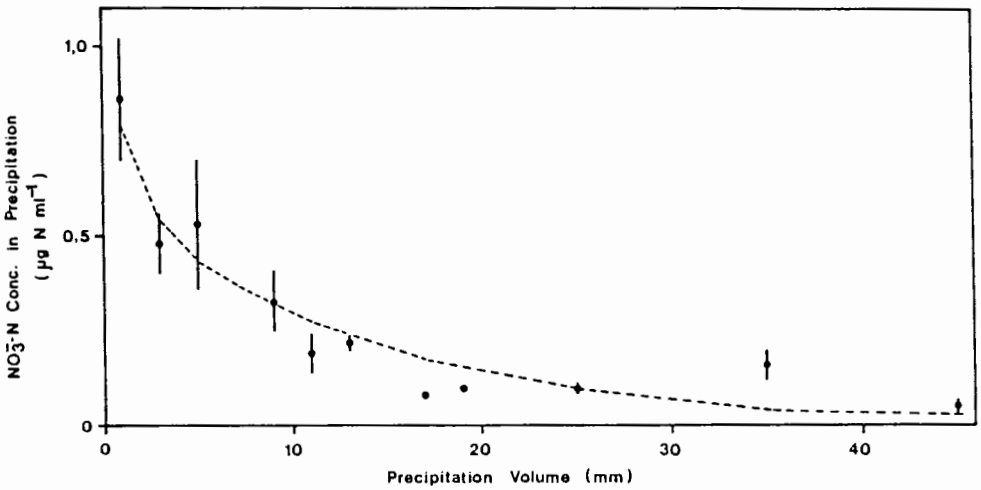

FIGURE 8.4: The relationship between NO_3-N concentration and weekly precipitation volume at Pella, South Africa. The logarithmic curve fit is y = a + bInx, where a = 0,79 and b = -0,21; r^2 = 0,90. The weekly precipitation volumes were grouped into classes and the mean $\pm$ SE of each class was calculated.

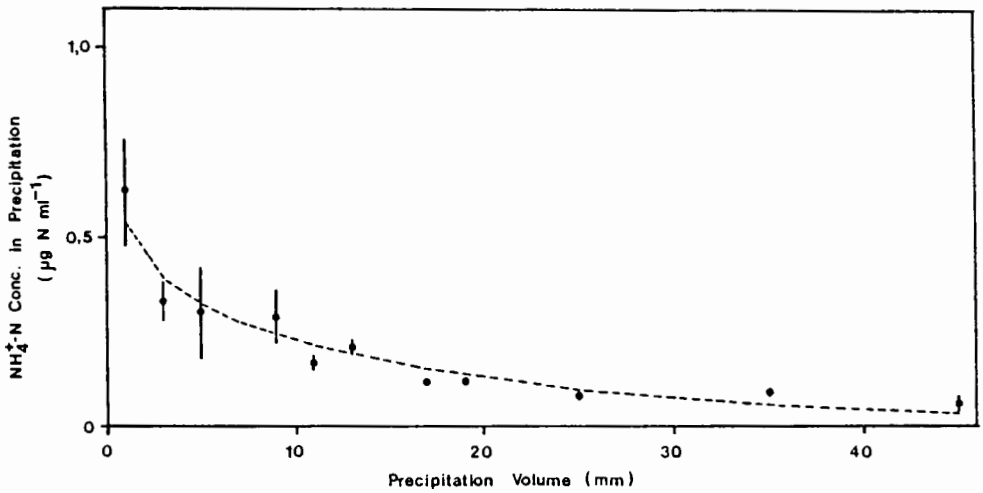

FIGURE 8.5: The relationship between NH_4-N concentration and weekly precipitation volume at Pella, South Africa. The logarithmic curve fit is y = a + bInx, where a = 0,55 and b = -0,14; r^2 = 0,92. The weekly precipitation volumes were grouped into classes and the mean $\pm$ SE of each class was calculated.

for the 1981-82 year was 35,0%, 25,2%, 21,4% and 18,4% for spring, autumn, winter and summer respectively. Logarithmic relationships exist between NO_3-N and NH_4-N concentrations in bulk precipitation and the volume of precipitation collected at weekly intervals (Figs. 8.4 & 8.5). The inorganic N input to collectors positioned at heights of 1,5 m and 4 m was approximately the same. A linear relationship between NO_3-N collected at 1,5 m and 4 m existed (Fig. 8.6). This was also the case with NH_4-N samples collected at 1,5 m and 4 m (Fig. 8.7).

DISCUSSION

Long-term measurements of precipitation conducted by the South African Department of Agriculture and Technical Services at the Burgherspost Farm (33° 30'S : 18° 32'E) (adjacent to the Pella site) show a mean annual rainfall of 577 mm. During the years studied (1980-82) we recorded below average rainfall values of 381 mm and 466 mm respectively with predominately winter rainfall (Fig. 8.1).

The input of total nitrogen from bulk precipitation at Pella (1,99 kg $ha^{-1}y^{-1}$) reported in this paper is low when compared with inputs to heathland and upland peat ecosystems of the northern temperate latitudes (8,7-19 kg N $ha^{-1}y^{-1}$, Carlisle *et al.* 1966, Allen *et al.* 1968, Gore 1968). Eriksson (1952) attributed the higher N

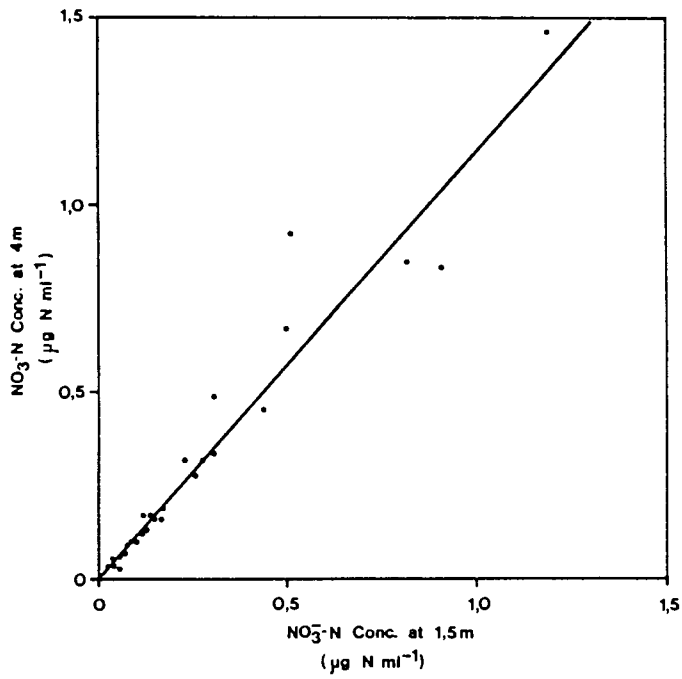

FIGURE 8.6: The relationship between nitrate concentration in precipitation collected at 1,5m and 4m above ground level at Pella, South Africa. The linear regression is y = a + bx where a = - 0,001 and b = 1,154; r^2= 0,95; n = 28.

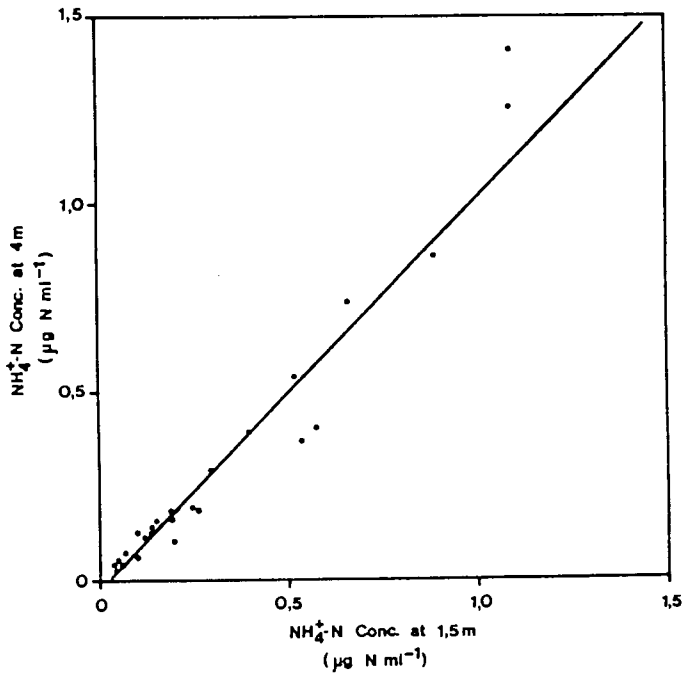

FIGURE 8.7: The relationship between ammonium concentration in precipitation collected at 1,5m and 4m above ground level at Pella, South Africa. The linear regression is y = a + bx where a = -0,031 and b = 1,050; r^2 = ,95; n = 28.

inputs found in Europe to the heavy industrial activity found in that area. The closest large industrial area which might influence N input at Pella is situated at Cape Town, 62 km south of Pella. Possible inputs from this source require southerly winds which are infrequent as the prevailing winds in summer are south-easterlies, and in winter north-westerlies.

The annual input of inorganic nitrogen, NO_3-N and NH_4-N in bulk precipitation for the years studied showed an increase as the annual rainfall increased (Table 8.1). The annual input of inorganic N (1,12-1,79 kg $ha^{-1}y^{-1}$) was similar to the input recorded by Schlesinger et al. (1982) in the Chaparral near Santa Barbara, California (1,5 kg N $ha^{-1}y^{-1}$) which is low when compared with inorganic N inputs to European ecosystems (4,5-7,0 kg N $ha^{-1}y^{-1}$, Gore 1968, Allen et al. 1968). Autumn and winter are the major seasons of inorganic N input and it would thus appear that the source of N contaminants is the South Atlantic Ocean, rather than terrestrial, as these seasons are characterised by the oceanic, north-westerly rain-bearing winds. Brown et al. (1984) have suggested that phosphorus input to this ecosystem is also likely to be from oceanic sources. Van Wyk (1982) found that in the mountain catchments of the South Western Cape, high concentrations of Na^+ and CI^- indicated the relative importance of the sea as a source

of dissolved nutrients in precipitation.

The mediterranean-climate and sandy soils of the study area give rise to long periods of soil drying followed by atmospheric suspension of soil dust by wind. This local source of atmospheric N does not appear to influence N inputs to any great extent as the quotient of soil NH_4-N to NO_3-N is approximately 2 (Stock & Lewis 1984b) while the quotient of NH_4-N to NO_3-N in the collectors over the two year study period remained at 1. Further, the location of collectors at 4 m (2,5 m higher into the ground level turbulence zone than the other collectors) did not reduce the input of N nor did the ratio of NO_3-N to NH_4-N change. This lack of influence from local sources indicated the importance of long-range atmospheric transport of particles from other environments.

The logarithmic relationship between NO_3-N concentrations and weekly rainfall input, and NH_4-N concentrations and weekly rainfall input (Figs. 8.4 & 8.5) showed that N derived from intra- and extra-system sources was progressively cleansed from the atmosphere with rainfall volumes up to approximately 8 mm. With rainfall volumes above this figure N concentrations did not increase and the N added to the system during intensive storms was probably predominately extra-system N from oceanic sources, as discussed above. The extent

of local recycling of N could not be separated from bulk input, thus the total and inorganic N inputs reported in this paper must be an overestimate of new nitrogen to the system.

Frequent fires are a characteristic feature of fynbos ecosystems (Moll et al. 1980) and losses of N by volatilization, surface runoff and dry erosion may deplete the system at a more rapid rate than it is replenished by atmospheric deposition and nitrogen-fixation. Van Wilgen and Le Maitre (1981) calculated from different aged fynbos stands (12 - 21 years old) that 20,4 - 158,5 kg N ha^{-1} was released when the vegetation was burnt. From the results reported in this paper it can be estimated that if all N released by fires is lost to the ecosystem, atmospheric deposition, as the sole N source, would take between 10 - 80 years to replace the nitrogen lost. This period is longer than the interval between fires (Moll et al. 1980). Therefore, although N deposition may contribute a significant portion of the extra-system N increment, it alone is not sufficient to replace N lost as a result of recurrent short-interval fires.

The low input of organic and inorganic N to the Pella site reported in this paper gives an indication of the natural levels of N deposition to be expected in the fynbos region of the South Western Cape where N in bulk

precipitation is derived mainly from oceanic sources and few anthropogenic influences alter precipitation composition.

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CHAPTER 9

GENERAL DISCUSSION

The pattern of nitrogen cycling, together with that of the cycling of other nutrients, is an important factor in describing the functioning of an ecosystem. Investigations of processes important in N cycling in coastal fynbos are considered independently in chapters of this thesis because each section had discrete objectives and methodological problems of its own. In this chapter an attempt is made to combine the information presented in this thesis with existing literature so as to obtain an overall picture of N cycling in coastal fynbos.

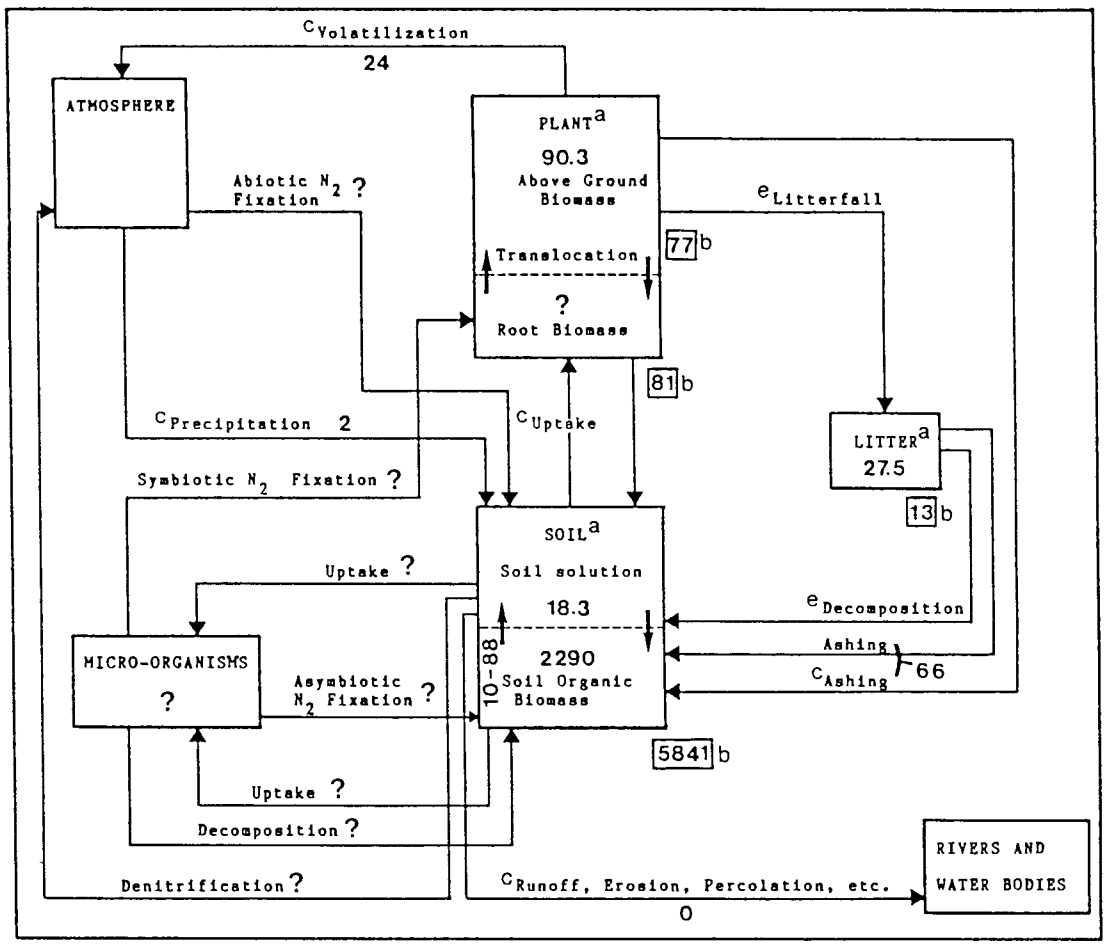
NITROGEN CYCLING IN COASTAL FYNBOS ECOSYSTEMS

Soil nitrogen reserves

The soils of the Pella study site are low in total and available N (total N and available N to 200cm depth, 2290-5840 kg ha^{-1} and 18,3 kg ha^{-1} respectively) and is similar to the low nutrient *Banksia* scrub soils in Australia (total N of 4280 kg ha^{-1} in surface 70 cm, Maggs & Pearson 1977). Soils from the other mediterranean regions of California, Chile (Thrower & Bradbury 1977, Mooney & Rundel 1979, Rundel & Parsons 1980) and the Mediterranean Basin have been shown to have much higher total N contents (*Quercus* garrigue ecosystem, South France has 6000 kg ha^{-1} in the surface 30cm, Lossaint & Rapp 1971). The low total N content of the Clovelly soil at Pella may be attributed to climatic influence (low annual rainfall, 577mm and high annual mean temperatures, 17°C) as well as the low clay

content of the parent material of soil formation (Table Mountain Sandstone and Cape Granite geological formations). The correlation of soil total N content with rainfall has been shown by Campbell (1983) in the montane fynbos environment.

Although the total N content of the soil at Pella is extremely low, it nevertheless still forms the greatest N reserve within the system (92% of 20 year old coastal fynbos reserves, Fig. 9.1; 97% of 11 year old coastal fynbos reserves, Low 1983). The available N pool in the Pella soil beneath 20 year old fynbos is only 0,8% of total soil N reserves and is predominately in the form of NH_4-N (Fig. 9.1). Total and inorganic N are concentrated at the soil surface, as expected, because this is where soil biological activity is greatest (Charley & West 1975). Seasonal changes in soil inorganic N concentrations revealed small significant monthly variations. No large quantities of either NO_3-N or NH_4-N were found to accumulate (Chapter 4), in contrast to the findings of Lossaint (1973) in a Quercus ilex ecosystem in the French mediterranean region. A lack of seasonal accumulation of mineral N is a result of a balance between inorganic N inputs to the soil from mineralization and the atmosphere and losses from plant uptake, leaching, microbial immobilization and denitrification. This delicate balance is indicative of an undisturbed natural ecosystem which has a very efficient "tight" nitrogen cycle.



a) The size of each N compartment was calculated from the phytomass data of O'Callaghan (1981) and the N concentration of a 21 year old fynbos stand at Jonkershoek (van Wilgen & Le Maitre 1981).

b) The size of N compartments in an 11 year old stand were obtained from Low (1983).

c) Nitrogen transfers were estimated from studies reported in this thesis except for the estimate of hydrologic losses which were based upon the results of van Wyk (1981).

d) Question marks indicate processes which have not been studied in fynbos ecosystems.

e) Processes which are being studied although no published data are available.

FIGURE 9.1: Major nitrogen compartments (kg N ha^{-1}) and pathways of nitrogen transfer (kg N ha^{-1} y^{-1}) in a 20 year old coastal fynbos ecosystem at Pella, South Africa. The size of N compartments in an 11 year old coastal fynbos ecosystem of the Cape Flats are shown in the boxes adjacent to the major compartments.

Soil nitrogen cycling processes

Investigations of the reserves of N in soils beneath coastal fynbos demonstrate the low N status of the ecosystem but provide little indication of rates of N turnover within the system or details of the dynamics of the mineralization process (Harmsen & van Schreven 1955, Ellenberg 1977). In agricultural systems N availability has been determined by a number of chemical and biological methods, including one where inorganic N release over a period during which the soils were incubated under controlled conditions was applied (Stanford & Smith 1973, Smith et al. 1980). This technique has been successfully applied to soils from natural ecosystems such as the chaparral and has enabled researchers to produce simulation models which provide estimates of N released (Marion et al. 1981). Using an incubation method mineralization of N in fynbos soils was shown in this study to be relatively slow (0,92 mg N g^{-1} soil organic N d^{-1} under optimum conditions of field capacity water content and a temperture of 30°C). Both NO_3-N and NH_4-N accumulated during the incubation of soils under controlled conditions, although NO_3-N predominated in soils incubated for more than 28 days (Chapter 5). Potential mineralizable N content in fynbos soils is low and of the order of 3,8% of total soil N which is similar to the 0,2-4,4% of total N reported by Marion et al. (1981) in chaparral soils. The estimate for annual N release from mineralization is about 88 kg h^{-1} if the total soil 2m soil depth is considered, however mineralization activity is generally limited to the better aerated surface layers and therefore a more

realistic estimate is 10 kg ha^{-1} which would be released in the surface 10 cm.

As the mineralization process is a dynamic one it appears that patterns of inorganic N release by mineralization change with the successional age of the stand, because the quotient of NH_4-N to NO_3-N found *in situ* changes in soils beneath fynbos at different seral stages. Odum (1969) hypothesised that the efficiency of nutrient use in ecosystems intensified as ecological succession proceeded, which according to Rice and Pancholy (1972) could result from the inhibition of the nitrification step of the mineralization process by plant produced allelochemicals. Reduced losses of N would occur as there is less NO_3-N for leaching, and energy is conserved by the plant as it does not require a reduction step during the assimilation of N. The results of the mineralization study reported in this thesis indicate that nitrification is not inhibited during the course of succession. The predominance of NH_4-N over NO_3-N *in situ* appears to be related to environmental controls favouring the ammonification rather than the nitrification process. The lag phase of 14 days before detectable nitrification occurs (see incubation experiments, Chapter 5) and the greater sensitivity of this process to lower water potentials, temperatures and low soil pH values than the ammonification process results in no large scale nitrification occurring because environmental conditions controlling nitrification change rapidly in the sandy, freely draining soils of the Pella site. The ammonification process, however, is less

sensitive to the environmental changes and does not have the same lag phase before NH_4-N begins to accumulate. The mineralization process controlled by environmental factors peculiar to the Mediterranean climate is probably the rate-limiting process controlling N turnover and potential productivity of undisturbed coastal fynbos ecosystems.

The soil N research reported upon in this thesis, and the existing literature available on fynbos ecosystems has been concerned mainly with N reserves within the soil and the mineralization process. Other processes are nevertheless important in controlling soil N reserves and N turnover through the soil system. These processes require examination before a more complete soil N cycle can be produced. Processes of particular importance in this regard are: asymbiotic N_2 fixation, denitrification and leaching of different N forms in the sandy soils characteristic of coastal fynbos ecosystems.

Litter nitrogen reserves

O'Callaghan (1981), Low (1983) and van Wilgen and Le Maitre (1983), as part of their phytomass studies of fynbos vegetation, showed the quantity of N in the litter component to vary with the size of the litter phytomass and the age of the community. Coastal fynbos litter mass was found to vary from 4503 to 9185 kg ha^{-1} at Pella in 8 year and 20 year old stands (Mitchell, Coley, Webb & Allsopp pers comm.*, O'Callaghan 1981) while Low (1983) found a litter mass of 2730 kg ha^{-1} beneath 11 year old coastal fynbos. The N reserves in the litter layer of a 20

*Mitchell, D.T., Coley, F., Webb, S. & Allsopp, N., Department of Botany, University of Cape Town, South Africa.

year old stand at Pella was estimated to be 27,5 kg N ha^{-1} while Low (1983) showed 13 kg N ha^{-1} to be contained in the litter of an 11 year old stand.

Litter nitrogen cycling processes

The dynamics of the litter layer in coastal fynbos is being studied (Mitchell, Coley, Webb & Allsopp pers comm.*) but as the return of nutrients is not a simple process to interpret over a few seasons, no published data are available as yet. In other mediterranean ecosystems Specht (1981) was able to show that N is only released from litter during decomposition in the 3rd year after litterfall. Schlesinger and Hasey (1981) demonstrated that N increased in the first year of decomposition in a chaparral stand. Even without a detailed knowlege of the complete decomposition process the investigation of the mineralization step of decomposition allows us to predict that the decomposition of sclerophyllous and woody material in coastal fynbos ecosystems is very slow and the litter which accumulates with the age of the stand makes this a fire-prone environment.

Vegetation nitogen reserves

Although not the largest part of ecosystem reserves, the N content of the plant compartment (3-10% of total N in ecosystem, Fig. 9.1) reflects the biological activity of the system. Annual primary productivity and phytomass at any site within the fynbos biome is influenced by many factors including differences in climate, soil properties, species composition, site nutrient

*Mitchell, D.T., Coley, F., Webb, S. & Allsopp, N., Department of Botany, University of Cape Town, South Africa.

status and period from last fire. The size of the N reserves in vegetation depends upon standing phytomass, nutrient concentration in the main plant organs and the relative allocation of biomass to different vegetative compartments. Studies of vegetation phytomass and N reserves in fynbos communities have been attempted by O'Callaghan (1981), van Wilgen and Le Maitre (1981) and Low (1983) (Fig. 9.1, Table 9.1). These workers have found that their results are similar to those obtained by DeBano et al. (1977), Gray and Schlesinger (1981) and Schlesinger et al. (1982a) in chapparal systems where it was demonstrated that total phytomass increases and average N concentration decreases with the age of the stand. In the study reported in this thesis phytomass and N reserves in the vegetation were not examined. The emphasis of the plant studies was rather on the problems of nitrogen nutrition of plants occurring in this mediterranean climate zone on soils of low nutrient status.

Plant nitrogen cycling processes

Recent reviews of how plants cope with a low nutrient environment have been provided by Chapin (1980), Clarkson and Hanson (1980) and Bowen (1981). These authors have enumerated a number of characteristics which may have evolved in species of low nutrient environments. These characteristics may include adaptations to improve nutrient uptake, reduce annual nutrient requirement and minimize nutrient loss.

In addition to plants such as Erica bauera (Ericaceae) maximizing absorptive surface area by mycorrhizal infections

TABLE 9.1: Phytomass, nitrogen content and nitrogen concentration of the plant compartment of some coastal and mountain fynbos ecosystems in the S W Cape Province, South Africa.

Ecosystem, Locale and Reference	Stand Age (years)	Phytomass (kg ha^{-1})	N Content (kg ha^{-1})	N Conc. (%)
Coastal fynbos, Cape Flats (Low 1983)	11	14 580	77	0,53
Coastal fynbos, Pella (O'Callaghan 1981)	20	30 090	-	-
Mountain fynbos, Zachariashoek (van Wilgen & Le Maitre 1981)	12	5 580	24	0,43
Mountain fynbos, Jonkershoek (van Wilgen & Le Maitre 1981)	21	35 420	105	0,30
Mountain fynbos, Bakkerkloof (van Wilgen & Le Maitre 1981)	12	4 190	18	0,43

(Read 1978), fynbos plants utilize finely-divided root systems such as those found in the Proteaceae (proteoid roots), Restionaceae (capillaroid roots) and Cyperaceae (dauciform roots) for this purpose (Lamont 1983). It appears that ericaceous mycorrhiza assist the host in exploiting low nutrient environments, not only by increasing absorptive area but by storing nitrogenous products and releasing them during periods of stress (Stribley & Read 1976). Proteoid roots have been shown to have a greater nutrient uptake, in particular phosphorus, from solution per unit mass than unmodified roots of the same species (Jeffrey 1967, Malajczuk & Bowen 1974, Lamont et al. 1984, Mitchell & Allsopp 1984). Increased uptake of N by these specialized root systems has not been demonstrated although Read and Mitchell (pers comm.*) have found proteoid roots of Protea repens and Hakea sericea to contain a protease enzyme which may enable the plant to utilize soil organic nitrogen directly, thus enhancing N uptake. The N physiology of other specialized root systems in the fynbos, namely the capillaroid and dauciform forms, have not been studied.

Very low growth rates and an evergreen perennial habit, which are characteristic features of coastal fynbos species, are among the principal features of plants growing in low nutrient environments (Beadle 1966, Small 1972, Grime 1979). Lewis and Stock (1978) found that leaves of an evergreen perennial member of the Proteaceae, Leucadendron xanthoconnus were unable to utilize the high levels of nitrogen supplied to them by petiole feeding, and it was concluded that this species had a low

*Read, D.J. & Mitchell, D.T., Department of Botany, The University, Sheffield, United Kingdom.

metabolic activity attuned to the low nutrient status of the environment. Futher studies of the nitrate reducing capabilities of South African members of the Proteaceae (P. repens and P. cynaroides) show shoot and root nitrate reductase activity to be low when compared with nitrophilous annual plants such as Hordeum vulgare (Chapter 3, Section 3.2.4). Uptake and assimilation rate of ^{15}N supplied either as NO_3-N or NH_4-N revealed small responses by P. repens to N addition and a slight preference for NH_4-N over NO_3-N absorption was noted (Chapter 6). Thus the Proteaceae exhibit a range of mechanisms to overcome nutrient limitation which enables them to survive and speciate in nutrient poor ecosystems.

Plant adaptations that minimize nutrient loss such as reduced leaching from the plant, effective nutrient retranslocation from senesced material and internal storage of N can futher enhance the success of plants growing in low nutrient environments. Sclerophyllous leaves with a well developed cuticle, characteristic of fynbos species, and low N contents of the vegetation make the plants less prone to leaching losses. Mooney et al. (1977) suggested that in plants possessing an asynchronous organ growth pattern with an efficient system of internal cycling of nutrients, the same nutrient capital can serve several functions during the growing season thereby overcoming nutrient limitations. In the study of Thamnochortus punctatus, an endemic member of the Restionaceae, the asynchronous organ growth pattern apparently allowed for highly efficient use of existing plant N capital (Chapter 7). This

member of the Restionaceae was also shown to have a highly efficient mechanism for the conservation of plant N capital as it withdrew between 35-70% of culm and branch N at senescence.

The adaptations discussed, which increase effective nutrient cycling in low nutrient environments, are probably essential for the continued survival and conservation of existing fynbos ecosystems. With the high species diversity of the fynbos ecosystem more studies of the range and degree of specialization of species to the low nutrient soils need to be undertaken before predictions concerning the stability and resiliance of the vegetation to disturbance can be attempted.

A neglected field of research concerning adaptations to low nutrient environments in fynbos ecosystems is an evaluation of the number of symbiotic N_2 fixing species and the importance of this process in coastal fynbos functioning.

Atmospheric nitrogen inputs and other inter-system cycling processes

The input of N to coastal fynbos from atmospheric deposition possibly constitutes an important source of N to replenish that lost by volatilization during recurrent wildfires in an ecosystem where the soils are of a low N status. Few estimates of N contributions from bulk precipitation sources in fynbos ecosystems have been undertaken. Van Wyk (1982) found no evidence of N in bulk precipitation in the Zachariashoek (33°41'S:19°06'E) and Jonkershoek (33°56'S:19°00'E) water catchment areas. In other mediterranean ecosystems, however, N has been detected in

precipitation and Schlesinger et al. (1982b) recorded an inorganic N input to a chaparral ecosystem near Santa Barbara, California, of 1,5 kg N ha^{-1} y^{-1}. This is low when compared with the input to European ecosystems (4,5 to 7,0 kg N ha^{-1} y^{-1}, Allen et al. 1968, Gore 1968).

The study reported in this thesis (Chapter 8) showed that, contrary to van Wyks' evidence, a detectable input of organic and inorganic N to a fynbos system at Pella was evident from precipitation (1,12 to 1,79 kg inorganic N ha^{-1} y^{-1} and 1,99 kg total N ha^{-1} y^{-1}). The total N input to the system was low, and very similar to that found in chaparral (Schlesinger et al. 1982b). Most of the N in bulk precipitation in the South Western Cape appears to be derived from oceanic sources and few industrial or other anthropogenic influences alter precipitation composition.

The significance of the low quantities of inorganic N input from precipitation in plant nutrition in the S W Cape is unknown, but if between 10 to 80 kg inorganic N ha^{-1} are released by mineralization each year, as has been shown in this study, then this N input in bulk precipitation could contribute up to 17% of the annual quantity of N made available to plants (Fig. 9.1).

Other intersystem processes (processes which link an ecosystem to other systems, Duvigneaud & Denaeyer-De Smet 1970; cf. intrasystem processes) affecting the N cycle, such as runoff, erosion, percolation and leaching, have been studied in mountain fynbos water-catchments of the S W Cape. Van Wyk (1982) has shown that in streams draining the Jonkershoek and Zachariashoek

catchments the nutrient losses from fynbos ecosystems are very low and that in the case of most nutrients input from precipitation exceeds hydrologic output. Again van Wyk (1982) failed to detect N compounds in drainage water and he suggests that losses of this element by this avenue are exceptionally low. Results of inorganic N determinations in the soil profile at Pella (Chapter 4) do not show any significant movement of N down the profile. It may be predicted, therefore, that the tight nutrient cycle of mature fynbos efficiently recycles all available N with little drainage losses depleting the N reserves of the system.

Fire and fynbos nitrogen cycling processes

Fire is the most common natural and man-made disturbance (accidental or intentional in origin) in fynbos vegetation and is considered to have been an evolutionary feature of the biome (Moll et al. 1980). Studies of wildfires in the South Western Cape have concentrated on producing a fire management program suitable for this fire-prone environment (Kruger 1981, van Wilgen & Kruger 1981, van Wilgen 1984a&b). As in other mediterranean regions, studies have concentrated on understanding the effects of fire characteristics (including intensities, seasonalities and frequencies of fires) on community dynamics of the local vegetation (survival, reproduction and succession) (Jordaan 1981, Parsons et al. 1981, Bond et al. 1984). Ecophysiological studies, including nutrient cycling studies, have not been utilized for management planning in fynbos ecosystems. Recently Dunn and

DeBano (1977) have documented a shift in research emphasis from physical and hydrological aspects of chaparral ecology to one concerned with nutrient cycling. They suggest that an understanding of nutrient cycling within an ecosystem provides a valuable tool which the land manager can use to test and manipulate vegetation growth and succession to achieve various management goals. They further highlighted the N cycle as needing special attention because of this elements potential to be lost to the system by volatilization during fires and the possible replenishment of the element by N_2 fixing organisms.

A change in soil N quantity and form is usually a good indicator of the severity of an ecosystem perturbation such as fire, clearfelling or tilling (Likens et al. 1970, DeBano & Conrad 1978, Doran 1980a&b, Rundel 1983). These parameters are good indicators of disturbance because the response of the system to the disturbance is, to a large extent, determined by the capacity of the soil to retain or release N (Heal et al. 1982).

Studies of the effect of fire on soil N at Pella showed that fire is a very effective, if destructive, mineralization process which causes a release of available nutrients into the system (Chapter 4). The direct effects of a moderate fire were evident as increased post-fire concentrations of total N and NH_4-N at the soil surface. Of the pre-fire 117,8 kg N ha^{-1} contained in the vegetation and litter compartments, 66 kg N ha^{-1} was found to be added to the soil surface. As only 76% of the vegetation was consumed by the fire (O'Callaghan 1981) it was estimated that volatilization losses were approximately 24 kg

N ha^{-1} (20,4% of above-ground reserves, Fig. 9.1).

Other results from the study reported in this thesis show that the indirect effects of fire are important in the post-fire environment because it is only at this stage of ecosystem development that NO_3-N was found to accumulate in fynbos soils. This post-fire NO_3-N flush was similar to that reported in chaparral soils by Christensen (1973). Mineralization experiments showed that the NO_3-N flush was not due to the destruction of allelochemical nitrification inhibitors, but to changes in soil chemistry and environmental factors controlling the mineralization process. As far as management policies are concerned this is of utmost importance in ecosystem dynamics because this altered nutrient environment is important in determining the species composition of the post-fire succession. It is at this stage that further losses of nutrients might be incurred by leaching and other intersystem cyling processes because the tight nutrient cycling strategies of the later successional species are not yet established.

If the input of N from precipitation were the sole source of N it would take between 10 to 80 years to replace the N released by a single fire (20,4 to 158,5 kg N ha^{-1} released in fynbos fires of different intensities) (Chapter 8). The lack of studies concerning the significance of the N_2 fixation process in the fynbos biome makes predictions concerning the overall importance of bulk precipitation in fynbos N cycling extremely difficult.

Management implications of research findings

This account of nitrogen cycling in fynbos vegetation has integrated a number of discrete studies of N cycling processes in the mediterranean climate region of the South Western Cape. In the following section topics of interest to managers of fynbos vegetation are discussed with reference to coastal fynbos N cycling in order to highlight areas where the nutritional aspects of fynbos functioning can be utilized when evaluating different management options or policies.

1) The low N status of the soils of the coastal fynbos stand at Pella has been demonstrated and as this appears to be a characteristic feature of both coastal and mountain fynbos ecosystems, all management practices should, wherever possible, attempt to maintain the existing nutritional characteristics of fynbos ecosystems. This is essential for the conservation of fynbos as changes in ecosystem nutrient quantity and cycling patterns can lead to an alteration in the species composition, structure and functioning of the ecosystem.

2) A valuable tool in future management of fynbos ecosystems is the monitoring of soil N quantities and forms. The soil because it is the largest resevoir of N can serve as a valuable indicator of the severity of the effect of management practices, such as fire, bushcutting and tilling on ecosystem functioning.

3) The low quantities of N available in the soils and the predominance of NH_4-N over NO_3-N in soils beneath mature fynbos is an important factor in the management of water catchments in the S W Cape. An important feature of managing

areas used as water catchments relates to the quality of the water for drinking purposes, in particular nitrate levels in ingested waters. High concentrations of nitrate in drinking water have been linked with the occurrence of methaemoglobinaemia and the possible development of nitrosamine carcinogenesis. Under mature fynbos there appears to be little or no movement of NO_3-N down the soil profile and subsequently into drainage waters. It is only during periods of ecosystem disturbance that NO_3-N accumulates in the soil and could possibly be leached into drainage waters feeding catchments. The fynbos ecosystem as a whole is, however, very low in N and unless heavy fertilizer applications are undertaken on such vegetation in catchments, no problems of nitrate in streams feeding water reservoirs should be experienced.

4) Fire is the major management tool in the S W Cape and has a great impact on the N cycle. The tight nutrient cycling patterns found in mature stands of fynbos are disrupted because N is mineralized and volatilized by fire. Changes incurred by fire include potentially large losses of ecosystem N reserves and these changes are important in determining the post-fire successional sequences. The effects of fire characteristics such as fire intensity, season of burn, fire periodicity, stand age and fire duration on N cycling in fynbos vegetation are as yet unknown. These fire characteristics and their effect on N cycling processes require study before management policies concerning the optimum period between fires and season of burn for effective management of this vegetation type can be determined.

5) The main thrust of ecosystem research in the S W Cape has until recently concentrated on the conservation and protection of fynbos vegetation. Recently the economic importance of this vegetation type has been realized and now many of the indigenous species are being exploited as cut flowers. This change in emphasis has important repercussions on the nutrition of indigenous species as the management problem changes from one of ensuring the optimum nutritional environment for the survival of the vegetation type as a whole, to a policy where production of a limited number of economically important species becomes of overriding importance.

Studies undertaken on individual fynbos species have shown that these plants have many adaptations to improve nutrient utilization in this low nutrient environment. The adaptations may include modifications to increase N uptake and N conservation, as well as mechanisms which adjust plant metabolic requirements to the low nutrient environment. Based on the results reported in this thesis, fertilization of fynbos vegetation is not recommended as the fynbos species investigated do not appear to respond to N addition. Heavy fertilizer applications will probably elict large species composition changes in the flora, similar to those reported in Australia (Specht 1963), without resulting in economically significant increased bloom production of the desired species.

6) Atmospheric input of N in bulk precipitation is low and is of little significance in the nutrition of fynbos ecosystems. The European problem of acid rain does not appear to be of immediate

concern because of the limited industrial activity in the region. Monitoring of the different forms and quantities of N in wet and dry fall, however, may provide a valuable tool to determine the nutritional impact of increased human populations, agricultural activity and industralization in the S W Cape on the conservation of fynbos ecosystems.

CHAPTER 10

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CHAPTER 11

APPENDICES 1-3

APPENDIX 1.

Extraction of nitrate reductase from members of the South African Proteaceae

W.D. Stock and O.A.M. Lewis
Botany Department, University of Cape Town, Rondebosch

The inhibition of *in vitro* nitrate reductase activity in extracts of *Protea* spp. is shown to be due to polyphenolic constituents of the roots and shoots of the plants (which can be adsorbed by insoluble polyvinylpyrrolidone) rather than to the activity of endogenous proteases. The *in vitro* nitrate reductase activity in shoots of *Protea repens* and *Protea cynaroides* fed 2 mmol $dm^{-3} NO_3^-$ for 24 h prior to nitrate reductase extraction show a nitrate reductase activity of 2 – 4 μmol NO_2^- h^{-1} while the roots of *P. repens* show an *in vitro* nitrate reductase activity of 0,2 μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$. The low nitrate reductase activity of these plants possibly reflects their adaptation to growth under the low nutrient condition of the soils of the South Western Cape, South Africa.

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Die remming van *in vitro* nitraat reduktase aktiwiteit in ekstrakte van *Protea* spp. word toegeskryf aan die polifenoliese bestanddele van die wortels en lote van die plante (wat deur middel van polivinielpirrolidoon geabsorbeer kan word) eerder as aan die aktiwiteit van endogene proteases. Die *in vitro* nitraatreduktase aktiwiteit in die blare van *Protea repens* en *Protea cynaroides* wat vir 24 h met 2 mmol dm^{-3} NO_3^- gevoer is voor nitraat reduktase ekstrahering, is 2 – 4 μmol NO_2^- h^{-1} (g vars massa)$^{-1}$, terwyl die wortels van *P. repens* 'n *in vitro* nitraat reduktase aktiwiteit van 0,2 μmol NO_2^- h^{-1} (g vars massa)$^{-1}$ getoon het. Die lae nitraat reduktase aktiwiteit van hierdie plante is moontlik 'n weerspieëling van hul aanpassing by die lae voedingswaarde van die grondsoorte van die Suidwes-Kaap, Suid-Afrika.

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W.D. Stock and O.A.M. Lewis*
Botany Department, University of Cape Town, Rondebosch 7700, Republic of South Africa
*To whom correspondence should be addressed

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Introduction

Although it has been shown in ^{15}N studies by Lewis & Stock (1978) that nitrate can be used as a nitrogen source in the nutrition of the shoots of Proteaceous plants, it has not been possible to demonstrate the presence of nitrate reductase (NR), which is NADH-dependent, in the leaves and roots of the plants using *in vitro* techniques and conventional extracting media. The inability to obtain an active extract of nitrate reductase could be owing to a number of factors, e.g. the presence in the plant of phytic acids, tannins or hydrolytic and oxidative enzymes (Loomis & Battaile 1966) or simply low levels of nitrate reductase activity (NRA). Problems in extracting active NR from leaves of members of the Ericaceae (*Vaccinium angustifolium* and *V. macrocarpon*) has resulted in proposals that the enzyme is entirely absent from these plants (Townsend & Blatt 1966; Greidanus *et al.* 1972). It has subsequently been found in other members of the Ericaceae (*Leucothoe catesbaei* and *Rhododendron catawbiense*) that inhibition of NR activity in extracts was due to the presence in the plant of a galloyl ester-like compound similar in nature to tannic acid (Dirr & Barker 1973). Other plants such as barley and maize are thought to contain a proteolytic enzyme capable of inactivating the enzyme complex by acting on the NADH (NO_3^-) c R component (Wallace 1974; Lewis *et al.* 1982).

The present study investigates the protective effects of agents added against polyphenolic inhibition and proteolytic enzyme inactivation on *in vitro* NRA of extracts of shoots and roots of *Protea repens* and *Protea cynaroides*. Polyphenolic inhibitors of enzyme extracts have successfully been adsorbed by insoluble polyvinyl-pyrrolidone (PVP) (POLYCLAR AT, BDH) (Loomis & Battaile 1966) while others (Schrader *et al.* 1974; Sherrard & Dalling 1978; Lewis *et al.* 1982) have shown that casein in the extraction medium prevents proteolytic enzyme degradation of active nitrate reductase.

Materials and Methods

Plant material

Plants of *Protea repens* (L.) L. and *Protea cynaroides* (L.) L. which had been cultivated for eighteen months in pots in a sand peat mixture with no supplementary nitrogen fertilization were used in the experimentation. Twenty-four hours prior to nitrate reductase extraction the plants were

fed 200 cm^3 Long Ashton nutrient solution containing 2 mmol dm^{-3} NO_3^- (Hewitt 1966). Barley (*Hordeum vulgare* L. cv. Clipper) leaves were obtained from plants germinated and grown in a nutrient film technique trough using a Long Ashton nutrient solution containing 2 mmol dm^{-3} NO_3^-. Leaves from twenty-day old barley plants were used for all barley assays.

Nitrate reductase extraction

Nitrate reductase was extracted from the leaves and roots of fresh *Protea* spp. by grinding 1 g plant material in a chilled mortar and pestle at 4 °C with 12 cm^3 of one of the following extracting media:

(a) 0,1 mol dm^{-3} phosphate buffer pH 7,5; 1 mmol dm^{-3} EDTA and 1 mmol dm^{-3} dithiothreitol to which was added 1,5 g of insoluble polyvinylpyrrolidone (BDH, POLYCLAR AT) (Loomis & Battaile 1966)

(b) 2,5% soluble casein (BDH), 0,1 mol dm^{-3} phosphate buffer pH 7,5; 1 mmol dm^{-3} EDTA and 1 mmol dm^{-3} dithiothreitol (Lewis *et al.* 1982).

The extract was squeezed through a double layer of cheese cloth and the filtrate was centrifuged at 2000 *g* for 5 min at 3 °C. All extractions of barley material followed the method of Lewis *et al.* (1982). The efficacy of each NRA protectant was determined by measuring its ability to prevent the loss of nitrate reductase activity in barley leaf extracts when extracts of *Protea* leaf and root were added to them.

Nitrate reductase assay

The reaction mixture for the determination of NRA was as follows: 0,1 cm^3 of 1 mol dm^{-3} phosphate buffer pH 7,5; 0,1 cm^3 NADH (1 mg cm^{-3}); 0,2 cm^3 of 0,1 mol dm^{-3} KNO_3 and 0,2 cm^3 barley extract or 0,3 cm^3 *Protea* extract made up to a final volume of 2 cm^3 with distilled water.

In assays where the inhibition of barley NRA by the *Protea* extract was investigated, 0,2 cm^3 barley extract and 0,3 cm^3 *Protea* extract were added to the reaction mixture before it was made up to final volume. The samples were incubated at 27 °C for 15 min and the reaction terminated with 1 cm^3 of 1% (w/v) sulphanilamide in 1,5 mol dm^{-3} HCl and 1 cm^3 of 0,01% (w/v) N-(1-napthyl) ethylenediamine hydrochloride solution. Absorbance was read at 540 nm after 5 min. Samples containing casein required centrifugation at 2000 *g* for 5 min to remove the coagulated protein. Triplicate aliquots of extract were assayed in each experiment.

Results and Discussion

Casein as a NRA protecting agent for *Protea* extracts

It is evident from the results shown in Table 1 that casein does not protect NRA in extracts of *Protea repens* leaf and root material. No NRA could be detected in the *P. repens* extracts and, in addition, the casein protected extracts of *P. repens* inhibited nitrate reductase activity in casein protected barley leaf extracts. The leaf extracts of *P. repens* caused a greater inhibition of barley NRA than did the root extract of the same plant.

These results indicate that the inhibitor responsible for NRA inactivation in *Protea repens* extracts is not a proteolytic enzyme as is probably the case in barley, but some other factor that is distributed in greater quantities in the leaf than in the root.

Table 1 Inhibition of barley leaf nitrate reductase activity by extracts of *Protea repens* leaf and root tissue in the presence of casein. Mean ± SE

	Plant extract				
	Barley leaf	Barley leaf + *Protea* root	Barley leaf + *Protea* leaf	*Protea* leaf	*Protea* root
Protectant	Casein	Casein	Casein	Casein	Casein
NR activity in μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$	6,9 ±0,23	4,8 ±0,19	2,4 ±0,08	0	0
Percentage inhibition of barley extract	0	29,6	64,6	–	–

Table 2 Nitrate reductase activity of *Protea repens* extracts protected with PVP. Mean ± SE

	Plant extract			
	Barley[a] leaf	*P. repens* root	*P. repens* leaf	Barley leaf + *P. repens* leaf
Protectant of *Protea* extract	[a]	PVP	PVP	PVP[a]
NR activity in μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$	7,7 ±0,14	0,2 ±0,01	1,3 ±0,06	8,7 ±0,30

[a] Barley always extracted with casein

PVP as a NRA protecting agent for *Protea* extracts

The activity of nitrate reductase extracted from *P. repens* leaves (protected by PVP), barley leaves (protected by casein) and a mixture of the two is shown in Table 2.

From these results it is apparent that when PVP is utilized as a protectant, significant NRA in *Protea* leaves can be demonstrated. In experiments where *Protea repens* extract was added to barley leaf extract virtually no inhibition of barley NRA was detected (barley leaf/*Protea* leaf mixture exhibited 96% the activity of the sum of the two extracts assayed individually). These results demonstrate the effectiveness of PVP as a protection agent for the extraction of NR from *Protea repens* and indicate that the leaves of this plant have a low (less than 15% barley leaf NRA) but detectable nitrate reductase activity. The NRA of *Protea repens* root (PVP protected) is also shown in Table 2; this is approximately 10% the activity of the leaves of this plant.

The *in vitro* NRA of the leaves of a second species of *Protea, Protea cynaroides*, was investigated using PVP protection in the extract preparation. The results are shown

Table 3 Nitrate reductase activity of *Protea repens* and *Protea cynaroides* leaf material. Mean ± SE

	Plant extract	
	Protea repens leaf	*Protea cynaroides* leaf
Protectant	PVP	PVP
NR activity in μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$	2,2 ±0,05	3,7 ±0,01

in Table 3 and indicate that the shoots of this plant have very similar NRA to *Protea repens*.

The results of these experiments indicate that the inhibitor responsible for NR inactivation in *Protea* spp. is probably not a proteolytic enzyme as in the case of barley, but a polyphenolic constituent of the plant that is distributed in greater quantities in the leaf than in the root. It is well known that *Protea* spp. have a high content of polyphenolic compounds as they were once used widely in the Cape Province, South Africa, as domestic tanning agents (Wehmer 1931; Williams 1930). Shoot NRA in the Proteaceae is low (2 – 4 μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$ when compared with nitrophilous plants such as *Zea mays* (9 μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$, Sherrard & Dalling 1978), *Hordeum vulgare* (14,8 μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$, Lewis *et al.* 1982) and *Helianthus annuus* (24,7 μmol NO_2^- h^{-1} (g fresh mass)$^{-1}$, Kaiser & Lewis 1981) and is possibly an ecophysiological characteristic of those members of the Proteaceae which are restricted to the low nutrient soils of the South Western Cape, South Africa.

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AN EVALUATION OF SOME MANUAL COLORIMETRIC METHODS FOR THE DETERMINATION OF INORGANIC NITROGEN IN SOIL EXTRACTS

KEY WORDS: Nitrate, nitrite, ammonium, inorganic nitrogen determination, soil extracts.

W. D. Stock.

Botany Department,
University of Cape Town,
Rondebosch, Cape.
7700, South Africa.

ABSTRACT

A number of manual colorimetric methods for the determination of inorganic nitrogen in 1 M KCl soil extracts were investigated to find techniques that were inexpensive, rapid, versatile and suitable for laboratories with limited analytical equipment. Three colorimetric methods for NO_3^--N determination were evaluated and only the copperised/cadmium reduction technique suffered no significant interference from the Cl^- present in the extracting solution. A phenol-hypochlorite (Berthelot) procedure for NH_4^+-N determination and the Griess-Ilosvay method for NO_2^--N determination were both found suitable for N determination in 1M KCl soil extracts. The reliability and accuracy obtainable with the manual colorimetric methods described was shown to be comparable with that obtained from colorimetric analyses performed using an AutoAnalyser.

INTRODUCTION

Potentiometric, colorimetric and distillation methods for the determination of soil nitrate, nitrite, and exchangeable ammonium are all widely used in soil and water analytical laboratories and the limitations imposed by the expense, rapidity, versatility and precision of the individual methods are the deciding factors in selecting which methods are to be used in a particular laboratory [1,6,8,13]. A large range of soil inorganic N extractants are available [3,10,11] and the adoption of a single solution for the simultaneous extraction and later analysis of all inorganic N forms would greatly accelerate and simplify the task of routine N extraction and analysis.

Bremner[3] and Sahrawat[11] have reported 1M or 2M KCl solutions to be the most effective NH_4^+-N extractants, whilst NO_2^- and NO_3^- recovery does not appear to be greatly affected by this extractant. A further advantage of the KCl extract is that it may be stored for a number of days before analysis as no changes in NH_4^+, NO_3^-, or NO_2^- levels have been observed[3].

As distillation procedures are time consuming and potentiometric methods (notably the nitrate electrode) are prone to Cl^- ion interference (Orion Nitrate Electrode Manual) a number of manual colorimetric methods have been investigated to find methods suitable for large numbers of N determinations in a laboratory not equipped with an AutoAnalyser.

In this investigation recently described methods for nitrate analysis, i.e. Szechrome NAS reagent (Szechrome NAS reagent pamphlet), chromotropic acid (CTA)[14] and a copperized cadmium reduction technique (Cu/Cd)[2,6,7,17] have been assessed in relation to possible Cl^- ion interference as these methods are reported to be less sensitive to Cl^- than the phenol-

disulphonic acid method (PDA). Nitrite concentration was estimated by the Griess-Ilosvay method of diazotization with sulphanilamide and then coupled with N-(1-napthyl) ethylenediamine to form an azo dye[1,8]. Bremner and Shaw[4] have shown Shinn's modification of this technique to be satisfactory for nitrite determination in 1M KCl and 1M K_2SO_4 soil extracts. Ammonium was determined by a manual indo-phenol blue method (IPB, Berthelot's reaction) which is extremely sensitive but requires stringent control to maintain reproducibility when applied as a manual method[9].

Results obtained from a range of samples analysed using manual colorimetric methods for nitrate and ammonium determinations in 1M KCl extracts were compared with results from automatic analysis to show that the reliability and accuracy obtainable with manual methods is comparable with that obtained from the more expensive and sophisticated automatic analytical procedures.

MATERIALS AND METHODS

Soil Inorganic Nitrogen Extraction

Ten grams of fresh soil were added to 40 ml of 1M KCl and shaken for 1 h. The solution was filtered through Whatman No. 1 filter paper and the filtrate analysed for nitrate, nitrite and ammonium. Blanks were prepared by filtering 40 ml 1M KCl through filter paper and analysing the solutions for ammonium which is normally present on the filter paper.

Nitrate Determinations

a) Chromotropic Acid Method
To 3 ml of NO_3^--N standard prepared in distilled water or 1M KCl 7 ml of 0.01% chromotropic acid working solution were added (prepared as described by Sims[14]). The

colour development of the CTA-NO_3^- complex was read on a spectrophotometer at 430 nm after 10 min. Unknown concentrations were calculated from a standard curve constructed for nitrate values between 0.3 and 8.0 µg N ml^{-1}.

b) Szechrome NAS Reagent
To a 0.5 ml aqueous sample 2.5 ml of Szechrome NAS reagent were added. The mixture was agitated whereupon colour development was read at 570 nm after 5 min. The colour is reported to be stable for 1 h (Szechrome NAS reagent pamphlet). Nitrate concentrations in the soil extracts were determined from a standard curve constructed between 0.1 and 2.0 µg N ml^{-1}. Szechrome NAS reagent was prepared by adding 5g Szechrome NAS powder to 1 ℓ of a 1 : 1 concentrated sulphuric acid phosphoric acid mixture.

c) Copperized Cadmium Reduction Analysis
Nitrate reduction was performed by adding 2g weight of prepared Cu/Cd to 3 ml of soil extract containing 0.1 ml 1M $MgCl_2$ (to overcome any possible phosphate interference) and 1.9 ml of a 0.4 M NH_4Cl buffer adjusted to pH 9.6 with NH_4OH. The mixture was shaken for precisely 10 min whereupon a 1 ml aliquot was removed and NO_2^- determined by the Griess-Ilosvay method [2]. Batches of 15 samples, 3 blanks and 5 standards were run simultaneously. Nitrate concentrations were calculated from a standard curve constructed between 0.1 and 1.5 µg N ml^{-1}.

d) Automatic Analysis
Nitrate was determined in 1M KCl soil extracts with a Technicon AutoAnalyser utilizing a

copperized cadmium reduction column as described by Grasshoff [5].

Nitrite Determination

Nitrite was determined in the 1M KCl extracts by the Griess-Ilosvay method in which 1 ml of 1% (w/v) sulphanilamide in 1.5N HCl and 1 ml of 0.01% (w/v) N-(1-napthyl) ethylene HCl solution were added to 1 ml of the soil extract. Colour was allowed to develop for 10 min after which the absorbance was read at 540 nm[1]. NO_2^--N standards were prepared in the range from 0.1 and 1.0 µg N ml^{-1}.

Ammonium Determination

a) Manual Indo-Phenol Blue Determination.
To 2 ml of sample or standard the following reagents were sequentially added with Gilson Pipetman dispensors: a) 1.6 ml 10% (w/v) sodium potassium tartrate solution; b) 0.2 ml 0.16% (w/v) sodium nitroprusside solution; c) 0.4ml sodium phenate reagent prepared fresh each day by dissolving 50 g phenol in 250 ml 40% NaOH and diluting to 400 ml; d) 0.2 ml sodium hypochlorite with 5% available Cl^-. The reagents were mixed and the solution made up to 10 ml. After 20 min incubation in a waterbath at 40°C the solutions were cooled and the absorbance read within 10 min at 625 nm. Batches of 15 samples, 3 blanks and 5 standards were run simultaneously. Standards were prepared in the range 0.1 and 3.0 µg N ml^{-1}. The timing of all stages of reagent addition and incubation are critical for reproducible colour development[1].

b) Automatic Analysis.
Ammonia was determined in 1M KCl extracts with a Technicon AutoAnalyser using the indo-phenol blue method [5,15].

RESULTS AND DISCUSSION

In Figs 1 and 2 it is evident that the CTA and Szechrome NAS methods suffer interference from the high Cl^- levels present in the 1M KCl extracts. The sensitivity of both techniques is drastically reduced when assaying standards prepared in 1M KCl when compared with the same standard prepared in distilled water. The Szechrome NAS method appears to be less severely affected than the CTA method (Szechrome NAS standard curve is still linear, although the slope is reduced with a correlation coefficient r = 0.993).

Sims[14] investigated Cl^- interference of the CTA method by the addition of Cl^- in concentrations of up to 10 µg ml^{-1} and found no significant interference, but in 1M KCl solutions the Cl^- concentration greatly exceeds that tested by Sims (1971) and colour development is inhibited. The Szechrome NAS reagent is reported to be interference free at Cl^- concentrations below 1000 µg ml^{-1} (Szechrome NAS reagent pamphlet). The Cl^- concentration in the extracting solution (35453 µg ml^{-1}) exceeds this upper limit and interference is apparent (Figure 2). These two simple and rapid colorimetric methods are not suitable for nitrate determination in 1M KCl soil extracts.

In Table 1 colour development of NO_3^--N and NH_4^+-N standards prepared in 1M KCl and determined by manual Cu/Cd reduction and Indo-Phenol Blue methods respectively are shown. Both techniques suffer little interference from Cl^- and the standard curve constructed for each is linear with a coefficient of determination for the linear regression of the standard curves greater than r = 0.995 in both cases. These

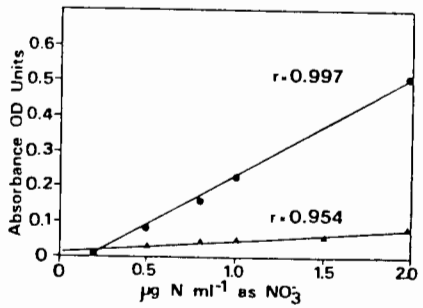

FIG. 1. Comparison of standard curves produced by colorimetric analysis of NO_3^--N using the chromotropic acid method when the standards are prepared in distilled water (●–●) and 1M KCL (▲–▲) .

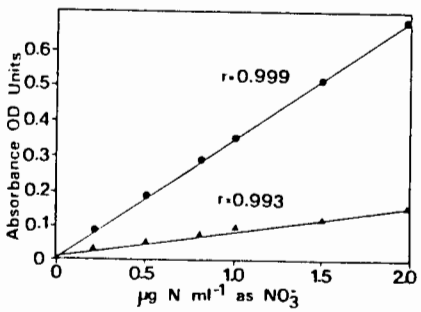

FIG. 2. Comparison of standard curves produced by colorimetric analysis of NO_3^--N using Szechrome analytical reagent when the standards are prepared in distilled water (●–●) and 1M KCL (▲–▲) .

TABLE I

Absorbance values obtained for the construction of standard curves for manual Cu/Cd reduction determination of NO_3^--N and indo-phenol blue determination of NH_4^+-N. Each absorbance value is the mean of 4 determinations for that concentration and the coefficient of variation (%CV) for each concentration is shown as well as the linear regression of N concentration versus absorbance.

Standard μg N ml^{-1}	Copper / Cadmium OD Units Mean	% CV	Indo-Phenol Blue OD Units Mean	% CV
0.1	0.063	4.2	-	-
0.2	0.131	2.7	0.023	11.5
0.5	0.317	0.7	0.082	2.7
0.8	0.503	1.4	-	-
1.0	0.626	1.3	0.215	1.9
2.0	-	-	0.460	1.3
3.0	-	-	0.674	1.6
	y = 0.004 + 0.623x		y = -0.026 + 0.236x	
	r = 1.000		r = 0.999	

methods have been utilized for inorganic N determinations in sea water and no Cl^- interference has been reported[6,17].

Manual colorimetric methods of inorganic N determination were compared with AutoAnalyser N determinations of the same samples to investigate the accuracy and reliability of these methods. It is apparent from Figs 3 and 4 that the results obtained from manual and automatic procedures are in close

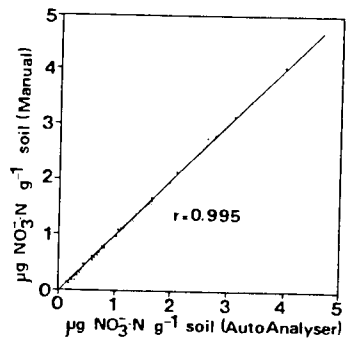

FIG. 3. Relationship between nitrate nitrogen concentration obtained in soil extracts when determined by manual and automatic copperized cadmium reduction techniques (n = 40).

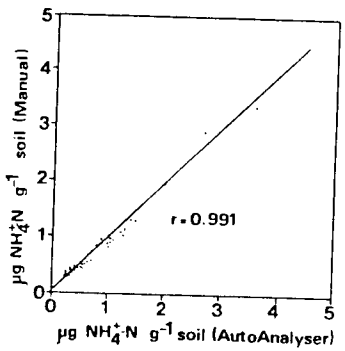

FIG. 4. Relationship between ammonium nitrogen concentration obtained in soil extracts when determined by manual and automatic indo-phenol blue procedures (n = 40).

agreement for both nitrate and ammonia determinations. In both cases the correlation coefficient for the linear regression of manual versus automatic nitrate and ammonium determinations was greater than 0.990.

Manual methods were performed at a rate of 60-80 nitrate or ammonium samples per person per day which enabled large numbers of samples to be analysed in a laboratory without the use of an AutoAnalyser.

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APPENDIX 3.

New Phytol. (1984) **97**, 261–268

UPTAKE AND ASSIMILATION OF NITRATE AND AMMONIUM BY AN EVERGREEN FYNBOS SHRUB SPECIES *PROTEA REPENS* L. (PROTEACEAE)

BY W. D. STOCK AND O. A. M. LEWIS

Botany Department, University of Cape Town, Rondebosch, 7700, South Africa

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SUMMARY

The uptake and assimilation of ^{15}N supplied either as NO_3^- or NH_4^+ has been studied over a 9-d period in *Protea repens*, a member of the Fynbos (South African Mediterranean-type vegetation) to establish the preferred N source for plant growth and development. Low uptake and assimilation rates were found for both NO_3^- and NH_4^+ feeding (4·8 and 5·9μg ^{15}N g^{-1} fresh mass d^{-1}, respectively) and total ^{15}N absorbed over 9 d was greater in the NH_4^+-fed plants than in NO_3^--fed ones. The form of N supplied did not cause significant changes in the total N content of the plants over the feeding period and no alteration in total N distribution patterns was evident, the shoot to root nitrogen ratio being 7:3.

Distribution of ^{15}N between the soluble N fraction and the bound fraction within root and shoot was similar for both NO_3^- and NH_4^+-fed plants. In both treatments approximately 70% of the ^{15}N was found in the root, mainly in the bound fraction.

Protea repens appears to have adapted to the conditions prevalent in the later stages of fynbos succession, not by showing an absolute requirement for a particular inorganic N form, but by an ability to absorb and assimilate small quantities of both NO_3^- and NH_4^+ which satisfy the low N demand of this slow growing, evergreen species.

Key words: Proteaceae, nitrate, ammonium, nitrogen assimilation.

INTRODUCTION

It is generally agreed that under natural conditions the main N sources for higher plants are NO_3^- and NH_4^+ (Haynes & Goh, 1978). Research on herbaceous crops which have evolved from ruderal species characteristic of nutrient-rich, disturbed sites has shown that each ion produces a different physiological response within the plant (Cox & Reisenauer, 1973; Reisenauer, 1978) and that plants differ in their ability to absorb and assimilate NO_3^- and NH_4^+ (Krajina, Madoc-Jones & Mellar, 1973; Lewis, James & Hewitt, 1982).

It has been proposed that nitrification in an ecosystem is inhibited by plant phenolics produced as succession proceeds (Rice & Pancholy, 1972, 1973), resulting in soils under climax vegetation having a low NO_3^- content and availability. The advanced secondary stages of fynbos vegetation of the South Western Cape Province of South Africa are dominated by members of the Proteaceae which are perennial, evergreen slow growing plants, and we have found that soils under mature (20 years old) *Protea repens* do, indeed, contain greater quantities of NH_4^+ than NO_3^-.

Members of this family appear to be adapted to the low N status of the soils by possessing a low rate of N metabolism which corresponds with a slow growth

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habit (Lewis & Stock, 1978). They also have a poor NO_3^- assimilation potential as reflected by low activities of nitrate reductase in the shoots and roots (Stock & Lewis, 1982); this finding suggests that members of the Proteaceae are suited to the later successional stages of the fynbos ecosystem by utilizing NH_4^+ as the predominant N source in association with a low N assimilation rate.

The objective of this study was to compare the uptake and assimilation of $^{15}NO_3^-$ and $^{15}NH_4^+$ in *P. repens* to establish whether there is a preferred N source for the growth and development of this plant and to investigate the sites and rates of NH_4^+ and NO_3^- assimilation in this species.

MATERIALS AND METHODS

Plant material

Seeds of *P. repens* (L.) were collected at the Fynbos Biome Research Site at Pella (33° 31′S:18° 32′E) after a wildfire. The seeds were sown on sand and 14 d after germination the seedlings were transplanted into pots containing acid washed sand. They were grown for 3 months in a well ventilated glasshouse and watered every 2 or 3 d with tap water until the cotyledons were chlorotic, indicating that all internal nutrient reserves had been utilized. The plants were then watered every 3 d with 50 ml of a one-tenth dilution of standard Long Ashton nutrient solution containing a mixture of 0·1 mM NO_3^- and 0·1 mM NH_4^+ (Hewitt, 1966). After 14 d on this nutrient regimé the ^{14}N was leached out of the acid washed sand in the pots with distilled water. From the following day nine plants were fed with 50 ml of a one-tenth dilution of the Long Ashton solution containing 2 mM 99·8 A% ^{15}N KNO_3 (Prochem Ltd, Deer Park Rd, London, UK) at pH 5 and a further nine plants with the diluted standard Long Ashton solution at pH 5 containing 2 mM 99·8 A% ^{15}N NH_4Cl. Feeding was repeated at 2-d intervals and the pot leachate from the $^{15}NH_4^+$-fed plants was analysed every 2 d to ascertain that no nitrification had occurred. Harvests were made after 3, 6 and 9 d, three plants from each feeding regimé being sampled at each harvest and ethanol soluble and bound N compounds extracted for determination of ^{15}N enrichment. Results are expressed as mean values from the three plants sampled at any one time ± the standard error of the mean (SEM).

Extraction and preparation of samples for ^{15}N determination

On harvesting, the fresh mass of the plant organs was determined and the material killed in liquid N, homogenized in cold 80% (v/v) ethanol (1 g fresh mass in 50 ml ethanol) and ethanol-soluble N compounds allowed to extract for 24 h at 0 °C. The samples were then filtered through Whatman No. 1 filter paper. The filtrate containing the ethanol-soluble N was evaporated down to 10 ml under an airstream while the residue remaining on the filter paper was oven-dried at 80 °C prior to micro-Kjeldahl digestion to determine the quantity of bound N, and ^{15}N enrichment of the fraction.

The preparation of soluble N samples for ^{15}N atomic emission spectrophotometric analysis differed for NO_3^-- and NH_4^+-fed plants, and was as follows:

The 10 ml ethanol-soluble N fraction from the NO_3^--fed plants was passed through a 3 × 1 cm ion-exchange column ('Dowex' 50W-X8 Standard H^+, 100 to 200 mesh particle size, Dow Chemical Company, Michigan, USA) to separate NO_3^- from the organic + ammonium fraction as described by Atkins & Canvin (1971). The NO_3^- fraction was placed in a Markham semi-micro distillation unit,

0·3 g Devarda's Alloy and 0·2 g heavy MgO powder (previously heated to 600 °C for 2 h and cooled) added and the ammonia produced after steam distillation collected in 2 ml 0·02 M HCl and back titrated with 0·005 M NaOH using Tashiro's indicator (screened methyl red). The organic + NH_4^+ fraction of the NO_3^--fed plants was converted to NH_4^+ by micro-Kjeldahl digestion with 3 ml conc. N-free sulphuric acid and a selenium catalyst tablet followed by steam distillation with 12 ml 50% w/v NaOH. The ammonium was collected in 2 ml 0·02 M HCl and prepared for ^{15}N analysis as described above.

Ammonium in the ethanol-soluble sample of the NH_4^+-fed plants was separated from the organic N fraction by a 3 min distillation with 0·2 g MgO as the alkalizing agent. This short distillation with mild alkali prevented hydrolysis of the amides in the organic N fraction (Bremner, 1965). The distillate was collected and its ammonium content determined by titration.

The organic N fraction of the NH_4^+-fed plants was prepared for ^{15}N analysis as described for the NO_3^--fed plants. Each fraction converted into the NH_4^+ form was reduced in volume to ± 1 ml and portions oxidized with sodium hypobromite under vacuum to produce N_2-filled discharge tubes by the method of Faust (1967). ^{15}N enrichment was determined on a Statron NOI 4 atomic emission spectrophotometer (Packard Instruments International SA, Zurich, Switzerland) and the results calculated from a calibration curve constructed for standards between 0·5 and 50% ^{15}N enrichment. Bound N was determined after micro-Kjeldahl digestion (followed by steam distillation) of the ethanol-insoluble residues, ^{15}N enrichment being estimated on the concentrated distillate as described above.

Results

Uptake and assimilation of nitrate and ammonium

The N uptake and assimilation rates of young plants were 4·8 μg ^{15}N g^{-1} fresh mass d^{-1} for NO_3^--fed plants and 5·9 μg ^{15}N g^{-1} fresh mass d^{-1} for NH_4^+-fed plants (Table 1). There rates are low when compared with those of rapidly growing species from more fertile habitats (e.g. barley with an assimilation rate of 12 and 39 μg ^{15}N g^{-1} fresh mass h^{-1}, respectively for 2 mM hydroponically-grown NO_3^-- and NH_4^+-fed plants, Lewis & Chadwick, 1983).

The total quantity of $^{15}NH_4^+$ (50·2 μg ^{15}N g^{-1} fresh mass) absorbed and assimilated by *P. repens* over 9 d was greater than $^{15}NO_3^-$ uptake and assimilation (33·4 μg ^{15}N g^{-1} fresh mass) over the same time period (Table 1).

Internal distribution of total nitrogen

The mean N content for the 18 experimental plants was 2964 μg N per plant with a mean N concentration of 896·2 μg N g^{-1} fresh mass. The distribution pattern of N between root and shoot was the same for all plants irrespective of the N form in the feeding solution for the duration of feeding. The root contained 31% (range 27·3 to 32·4%) of the total plant N with the balance, 69% (range 67·6 to 72·7%), contained in the shoot (Fig. 1).

The fresh mass of the plants fed on the different N sources (Table 1) did not change over the short duration feeding period and no mass differences could be ascribed to either NO_3^- or NH_4^+ nutrition.

Sites of ^{15}N incorporation and accumulation

After 9 d the ^{15}N content of the $^{15}NH_4^+$-fed plants increased to a maximum of 197·0 μg ^{15}N per plant which constituted 5% of total plant N. ^{15}N content of

Table 1. *Total N concentration, ^{15}N concentration, ^{15}N uptake and assimilation rate and plant fresh mass of young plants of* Protea repens *after 3, 6 and 9 d feeding with either 2 mM NO_3^- or 2 mM NH_4^+ (mean of three plants ± SEM)*

Duration of feeding period (d)	Nitrogen source 2 mM $^{15}NH_4^+$			Nitrogen source 2 mM $^{15}NO_3^-$		
	3	6	9	3	6	9
N concentration (μg N g^{-1} fresh mass)	900·1 ±136·00	911·6 ±46·8	868·6 ±30·4	975·4 ±100·3	835·2 ±44·8	886·3 ±32·4
^{15}N concentration (μg ^{15}N g^{-1} fresh mass)	19·0 ±1·9	34·8 ±2·9	50·2 ±8·4	19·3 ±2·4	25·0 ±6·5	33·4 ±9·7
^{15}N uptake and assimilation rate (μg ^{15}N g^{-1} fresh mass d^{-1})	6·3 ±0·6	5·8 ±0·5	5·6 ±0·9	6·4 ±0·8	4·2 ±1·1	3·7 ±1·1
Plant fresh mass (g)	3·40 ±0·32	3·78 ±0·40	3·92 ±0·06	3·53 ±0·34	3·24 ±0·29	3·62 ±0·27

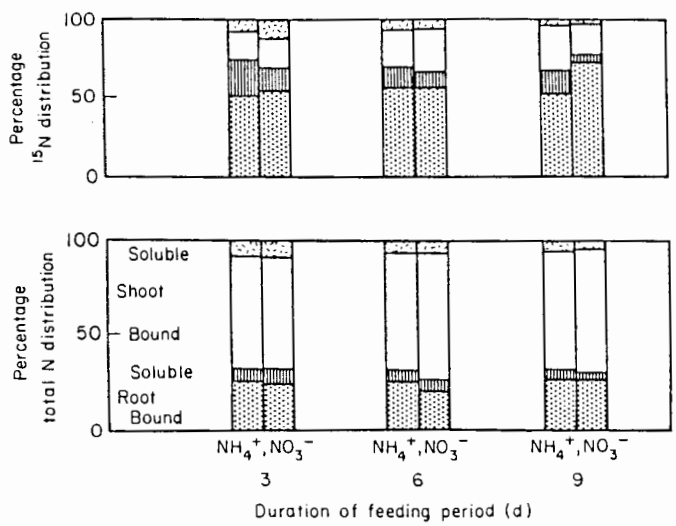

Fig. 1. Total N and ^{15}N distribution in *Protea repens* root-bound □, shoot-bound □, root soluble-▩ and shoot-soluble ▨ fractions after 3, 6 and 9 d feeding with either 2 mM $^{15}NO_3^-$ or $^{15}NH_4^+$.

the $^{15}NO_3^-$-fed plants did not increase to the same extent and reached a maximum of 123·4 μg ^{15}N per plant (3·9% of total plant N) (see Table 1).

Distribution of ^{15}N between the soluble and bound fractions within the roots and shoots was similar for both forms of N feeding with approximately 70% of the ^{15}N being incorporated in the root (Fig. 1). The major ^{15}N-containing fraction in the root was bound N which reached enrichment levels of 11·5 A%E ^{15}N and 9·6 A%E ^{15}N after 9 d for the $^{15}NH_4^+$- and $^{15}NO_3^-$-fed plants, respectively (Table 3). Bound ^{15}N in the shoots only attained maximum values of 2·7 A%E ^{15}N and 1·2 A%E ^{15}N, respectively, for the $^{15}NH_4^+$- and $^{15}NO_3^-$-fed plants over the same time period (Table 3).

The ^{15}N enrichment, size and content of the ethanol-soluble fraction in the roots

Table 2. *^{15}N enrichment, ^{15}N content of each plant part and ^{15}N concentration in the ethanol-soluble fraction of the roots and shoots of young plants of* Protea repens *after 3, 6 and 9 d feeding with either 2 mM NO_3^- or 2 mM NH_4^+ (mean of three plants ± SEM)*

Duration of feeding period (d)	Nitrogen source 2 mM $^{15}NH_4^+$			Nitrogen source 2 mM $^{15}NO_3^-$		
	3	6	9	3	6	9
Root						
Enrichment	8·3	6·4	14·0	4·5	5·4	6·5
A%E	±0·2	±0·3	±1·2	±0·7	±0·9	±1·4
^{15}N content	15·1	16·7	27·3	8·5	6·4	5·9
(μg ^{15}N per plant part)	±1·9	±1·8	±3·9	±1·1	±1·0	±1·4
^{15}N concentration	8·5	9·8	14·0	4·9	4·5	3·8
(μg ^{15}N g^{-1} fresh mass)	±1·5	±1·2	±1·7	±0·2	±1·2	±0·3
Shoot						
Enrichment	2·1	3·0	3·9	3·9	3·3	2·6
A%E	±0·6	±0·6	±0·8	±1·0	±1·0	±0·8
^{15}N content	5·0	8·3	8·6	8·1	4·6	3·6
(μg ^{15}N per plant part)	±1·7	±2·2	±1·7	±0·5	±1·4	±1·3
^{15}N concentration	3·2	3·8	4·3	4·5	2·8	1·6
(μg ^{15}N g^{-1} fresh mass)	±1·1	±0·7	±0·7	±0·8	±1·0	±0·5

Table 3. *^{15}N enrichment, ^{15}N content of each plant part and ^{15}N concentration in the bound N fraction of the roots and shoots of young plants of* Protea repens *after 3, 6 and 9 d feeding with either 2 mM NO_3^- or 2 mM NH_4^+ (mean of three plants ± SEM)*

Duration of feeding period (d)	Nitrogen source 2 mM $^{15}NH_4^+$			Nitrogen source 2 mM $^{15}NO_3^-$		
	3	6	9	3	6	9
Root						
Enrichment	4·3	8·6	11·5	4·9	5·9	9·6
A%E	±0·6	±1·2	±0·9	±0·5	±1·0	±2·9
^{15}N content	32·3	73·2	101·7	35·6	44·4	88·8
(μg ^{15}N per plant part)	±1·9	±15·8	±14·1	±2·7	±7·9	±20·6
^{15}N concentration	18·3	43·3	52·2	21·7	30·8	62·4
(μg ^{15}N g^{-1} fresh mass)	±2·6	±9·4	±6·1	±4·9	±7·6	±14·7
Shoot						
Enrichment	0·6	1·5	2·7	0·7	1·3	1·2
A%E	±0·1	±0·3	±0·5	±0·1	±0·4	±0·3
^{15}N content	11·3	31·9	59·3	14·1	22·6	25·0
(μg ^{15}N per plant part)	±2·2	±8·4	±16·5	±4·2	±7·5	±8·6
^{15}N concentration	7·2	15·7	29·8	7·7	13·5	11·2
(μg ^{15}N g^{-1} fresh mass)	±1·4	±3·6	±8·2	±1·7	±4·6	±3·5

and shoots (see Table 2) fluctuated over the 9-d feeding period as ^{15}N was absorbed, assimilated into the soluble fraction and then incorporated into bound N, where the ^{15}N enrichment continued to increase for the duration of the feeding period. No differences in root and shoot ^{15}N distribution patterns between $^{15}NO_3^-$- and $^{15}NH_4^+$-fed plants were apparent (Fig. 1).

Discussion

The capacity of higher plants to absorb and assimilate NO_3^- and NH_4^+ differs with species and these differences have been related to growth rates (Lee & Stewart, 1978; van de Dijk, 1980), soil pH (Havill, Lee & Stewart, 1974; Taylor & Havill, 1981) and N form and content of the edaphic environment (Lee & Stewart, 1978; Gigon & Rorison, 1972).

The results obtained in this study of young plants of *P. repens* indicate low uptake and assimilation rates for both NO_3^- and NH_4^+ (Table 1). This is consistent with the results of Lewis & Stock (1978) who showed a limited N assimilation capacity in another member of the Proteaceae, *Leucadendron xanthoconnus*. Other workers have shown that members of the Australian Proteaceae (e.g. *Banksia serrata*) cannot survive feeding regimés with high levels of P (100 μg ml^{-1} P) and N (250 μg ml^{-1} N in the ratio of 9 NO_3^- to 1 NH_4^+) whereas *B. serrata* grown with little or no fertilization with N and P could survive and grow (Groves & Keraitis, 1976).

Evidence from wild plants belonging to the family Ericaceae, which grow on acid soils, suggests that these plants show a preference for ammonium (Greidanus *et al.*, 1972) and it was at one time suggested that members of the Ericaceae were unable to reduce NO_3^- as the family were thought to lack nitrate reductase (Townsend & Blatt, 1966) but this suggestion has since been disproved by Dirr, Barker & Maynard (1973). Stock & Lewis (1982) demonstrated the presence of nitrate reductase in *P. repens* and the assimilation of $^{15}NO_3^-$ in this experiment provides further evidence (Table 1) of the ability of this species to reduce NO_3^-. The apparent slight preference shown by *P. repens* in this experiment for NH_4^+ uptake and assimilation might be due to NH_4^+ absorption being a passive process which generally occurs at rates greater than those observed for NO_3^- uptake (Higinbotham, 1973). In addition, the uptake and assimilation of NH_4^+ under natural conditions of low availability has the lowest assimilatory energetic requirement of any available N form (N_2, NO_3^- or NH_4^+) as no chemical transformation is required before plant assimilation (Gutschick, 1981). The major constraint on NH_4^+ uptake is the energetic cost of producing an increased root mass which is required to extract NH_4^+ which moves only short distances within the soil (Gutschick, 1981). The energetic constraint in producing root mass is overcome by the seasonal production of proteoid roots in the family Proteaceae (Lamont, 1982, 1983) which exploit a large soil volume, particularly pockets of organic matter immediately below the litter layer where rapid ammonification occurs.

Species differ widely in their ability to reduce and assimilate nitrate in the root. Wallace & Pate (1967) and Lewis *et al.* (1982) have shown respectively that both cocklebur and barley have low nitrate reductase activity in the roots with the leaves being the principal sites of NO_3^- assimilation, while Grasminis & Nicholas (1967) have shown that in nitrate-fed apple trees nitrate reduction normally occurs in the fine rootlets. Nitrate reduction in our experiment appeared to have occurred predominantly in the root as ^{15}N enrichment and ^{15}N content of the soluble pool and bound fraction of ^{15}N in the shoot were smaller than in the root even after 9 d (Tables 2 and 3). This is in spite of the findings of Stock & Lewis (1982) who demonstrated nitrate reductase activity to be greater in the leaves of *P. repens* than in the roots. In addition, Lewis & Stock (1978) demonstrated the occurrence of NO_3^- loading on to the xylem stream of other members of the Proteaceae

(*P. laurifolia* and *P. lepidocarpodendron*) for later reduction in the leaves of these species. It would appear that *P. repens* possesses the enzymes responsible for NO_3^- reduction and assimilation in both root and shoot and NO_3^- reduction may occur in both organs although in our experiment NO_3^- reduction appeared to occur mainly in the roots.

The assimilation of nutrient ammonium apparently took place predominantly in the roots of *P. repens* as the soluble N fraction of the root was rapidly enriched with ^{15}N and the assimilatory pools had already reached saturation by the third day of ^{15}N feeding. Further, the ^{15}N enrichment of the soluble N fraction in the shoot was low compared with that of the root (Table 2) and the allocation of ^{15}N to the bound fraction of the root was much higher than to the bound N fraction of the shoot (Fig. 1).

As with an evergreen chaparral shrub *Ceanothus megacarpus* Nutt., (Gray & Schlesinger, 1983) *P. repens* shows a minimal response to N addition and growth of this fynbos plant appears to be similar to other species from infertile habitats which show growth to have little dependence on soil nutrient availability (Specht & Groves, 1966; Rorison, 1968; Chapin, 1980) as these plants have the capacity to continue growth using internal reserves after soil nutrients have been depleted.

Protea repens thus appears to have adapted to the conditions prevalent in the later stages of fynbos succession, not by showing an absolute requirement for a particular inorganic N form, but by an ability to absorb and assimilate small quantities of both NO_3^- and NH_4^+ to satisfy the low N demand of this slow growing, evergreen species.

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