

**THE DISTRIBUTION AND FUNCTIONING OF RHIZOSHEATHS AMONG
SOUTH AFRICAN GRASS SPECIES.**

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**A dissertation submitted to the Faculty of Science, University
of the Witwatersrand, Johannesburg, for the degree of Master of
Science.**

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DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University, nor has it been prepared under the aegis or with the assistance of any other body or organization or person outside the University of the Witwatersrand, Johannesburg.



Catherine Lara Bailey

Twenty sixth day of January, 1994.

THIS THESIS IS DEDICATED TO MY PARENTS,
BILL AND EDNA BAILEY,
IN APPRECIATION FOR ALL THEY HAVE DONE FOR ME.

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ABSTRACT

Rhizosheaths are sandy coatings covering the entire length of the roots of many grass species. They consist of a mass of sand and silica particles embedded in a mucilaginous layer and are matted together by a meshwork of prolific epidermal hairs.

A study of the phenomenon of rhizosheaths in South African grasses was undertaken. Aspects which were investigated include the occurrence of rhizosheaths among South African grasses and the influence of environmental conditions on the presence and extent of rhizosheath development. In addition, information was gained on their possible functions in terms of their contribution(s) to plant vigour, through their influence on nutrient and water uptake, particularly in low nutrient soils in arid areas.

An extensive survey of herbarium specimens was conducted at the National Herbarium in Pretoria. It was found that the presence of rhizosheaths is a genetically fixed trait, occurring in the majority of grass species in South Africa. The extent to which sheaths develop (the thickness of the sheath and the degree to which the soil particles are bound to the sheath), varies between, and sometimes within species.

Seeds of three sheath forming grass species which occur in South Africa, *Antheophora pubescens* Nees, *Digitaria eriantha* Steud and *Eragrostis pallens* Hack, were grown under different conditions of soil texture and different conditions of water and nutrient (nitrogen (N) and phosphorus (P)) availability. Sheath development was found to be more extensive the higher the sand (relative to clay) content in the soil. In addition, rhizosheaths developed to a greater extent in sandy soil with high water and nutrient availability. Therefore, the extent to which sheaths develop is a facultative response directly to sandy soil, rather than the resulting lower water and nutrient availability in this soil.

After studying a number of physiological and morphological factors of the individuals in the different water and nutrient treatments it appeared that rhizosheaths compensated for low water availability. In addition, sheaths appeared to compensate for low N availability when this low availability was a result of low soil water content.

The influence of sheath thickness on immobile nutrient ion uptake was investigated. This was achieved by stimulating within-species variation in sheath thickness and measuring the difference in P uptake. The individuals with thick sheaths extracted more P from the soil, and from a greater volume of soil, than individuals with thin sheaths. This was particularly evident in conditions of low P availability, thereby highlighting the important influence of thick rhizosheaths in soils with a low P status.

The microbial biomass (as indicated by the microbial N and carbon concentrations) in the sheath soil was compared to that in the bulk soil and the rhizosphere/outer rhizosphere soil of unsheathed/sheathed roots. From this study it was concluded that rhizosheaths influence the microorganisms in the soil adjacent to the root surface, since the rhizosheath soil had a significantly higher microbial biomass than the soil from the other regions.

From the study it was concluded that the presence of rhizosheaths may be a mechanism employed by certain grass species in order to enhance their ability to tolerate dry soil, which has low N and P availability. This mechanism may be a substitute for increased root production and root branching as well as increased mycorrhizal associations in plants in arid, low nutrient status soils.

PART I INTRODUCTION

CHAPTER 1

INTRODUCTION TO RHIZOSHEATHS.

1.1 PLANT ROOT SYSTEMS.

The angiosperms (flowering plants) are divided into two classes: the dicotyledons and the monocotyledons. The two classes differ in root and shoot morphology and anatomy. The differences pertaining to roots will be highlighted since this study deals with a specialized root morphology typical of certain monocotyledonous plants, namely in the family Poaceae. The main differences between the two classes is that dicotyledons produce a primary root which grows directly downwards, becomes the predominant root of the system (the tap-root), and gives rise to lateral roots. In monocotyledons the primary root is short-lived and the root system is fibrous, consisting of adventitious roots which develop from the stem and give rise to lateral roots. This form of root system, characteristically found in grasses (Poaceae), provides a larger surface area per total plant biomass than root systems characterised by a tap-root.

The depth to which the root system is able to penetrate the soil and spread laterally depends on several factors, including the moisture, temperature and texture of the soil. The shallowness of fibrous root systems and the degree to which they cling to soil particles make them especially good ground cover plants for the prevention of soil erosion.

Another difference between the two angiosperm classes involves the internal structure of the root. The distribution of cell types within the stelar region differs between the two classes.

Many root system modifications occur depending on the environmental conditions in which the plants grow. For example, pneumatophores are roots which are produced by certain dicotyledonous plants which grow in anaerobic muddy soil. These roots extend upwards out of the soil and provide adequate aeration for the plant. Another adaptation is for food storage and is commonly found in both monocotyledons and dicotyledons. The development of rhizosheaths around the roots of certain monocotyledonous species, mainly in the Poaceae and some in the Cyperaceae families, has been observed and may be an adaptation to certain environmental conditions. This study was initiated to investigate this phenomenon and the possible environmental influences on the development of these sheaths and any functional role(s) that they may have in terms of increasing plant vigour and tolerance to stressful environmental conditions.

1.2 RHIZOSHEATHS.

The "rhizosheaths" look like sandy coatings covering the entire length of each root (Figure 1.1). They have been described as thick soil cylinders formed by modifications of the rhizosphere (Atlas and Bartha 1987), hence the name "rhizosheath". They have also been referred to as "sand grain root sheaths" (Wullstein, Bruening and Bollen 1979; Marneweck 1990) due to the predominance of sand particles in the sheaths. These descriptions imply that the "sheaths" are separate from the roots, however, they have been shown to be attached to the stele region of living roots (Marneweck 1990).

Rhizosheaths consist of a mass of sand and silica particles matted together by a meshwork of prolific hair-like epidermal structures, mucilage (Marneweck 1990) and other products released from the roots (Wullstein and Pratt 1981) (Figure 1.2). Although at first it may appear that rhizosheaths could be the result of mycorrhizal associations, these have not been found to occur in rhizosheaths (Wullstein and Pratt 1981; Buckley 1982 and

Goodchild and Myers 1987). Therefore fungal hyphae do not contribute to sheath formation or functioning.

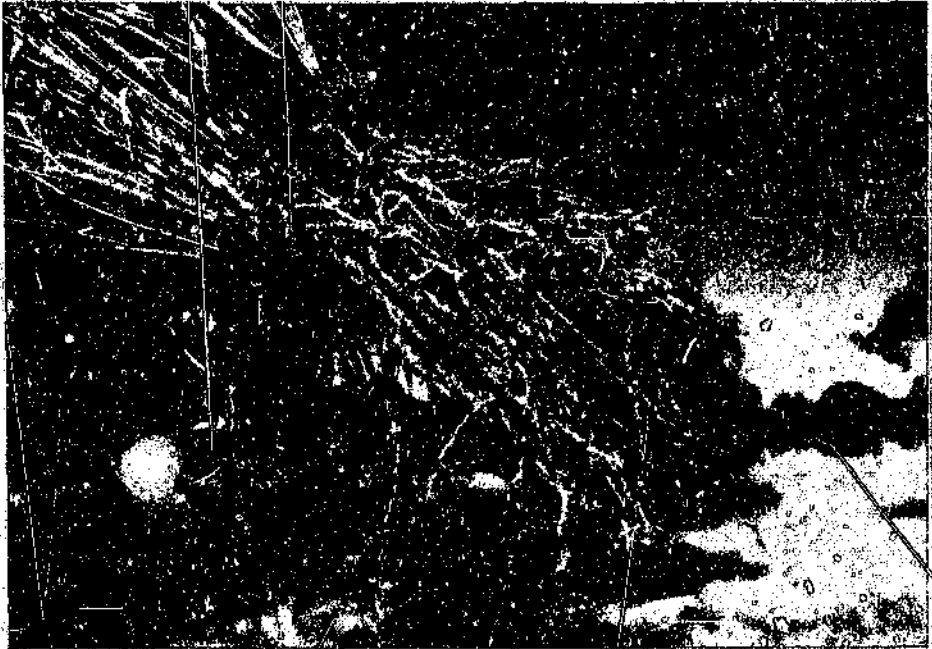


Figure 1.1. Thick rhizosheaths covering the entire length of each root of an *Eragrostis pallens* plant.

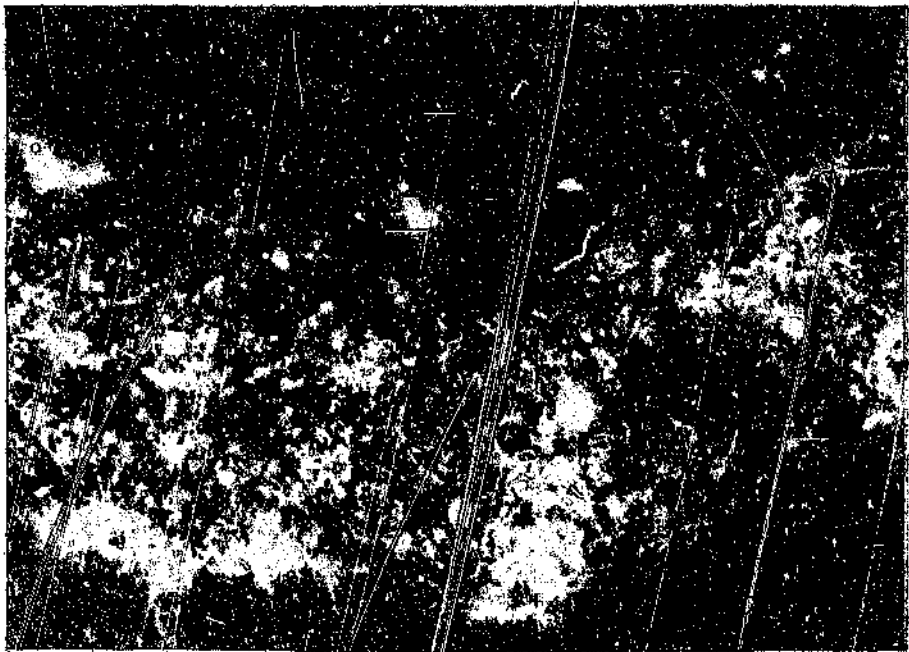


Figure 1.2. The mass of sand and silica particles matted together by the meshwork of epidermal hairs (a), mucilage and other substances released from the roots of *Eragrostis pallens*.

In dried specimens the cortex of the root disintegrates leaving a large gap between the stelar region and the epidermis. This results in the apparent sand grain "sheath" which is observed on dried grass specimens and which retains its shape even when the roots shrivel (Price 1911). Henrici (1929) reported that the cortex of certain xeromorphic grasses, remains intact for only a short distance behind the meristem. Beyond this the cells of the mid-cortex die and rapidly disintegrate leaving the outer cortical ring connected to the stele only by cell-threads composed of broken and collapsed cell walls. During these changes occurring in the roots, the sheaths maintain their strength.

Initially rhizosheaths were thought to be sand grains cemented together by root mucilage and that epidermal hairs functioned only in absorption (Price 1911). Later it was found that the epidermal hairs were directly involved in sheath formation (Oppenheimer 1960), primarily by mechanical bonding (Wullstein and Pratt 1981). This bonding appeared to be the main factor responsible for the sheath strength. Epidermal hairs, and often roots themselves, break easier than the bonding between the epidermal hairs and the sand grains. Therefore, it can be seen that grass species which develop rhizosheaths provide an even more suitable ground cover for the prevention of soil erosion than other monocotyledon species with fibrous root systems.

1.3 DISTRIBUTION OF RHIZOSHEATH FORMING GRASS SPECIES.

A review of the literature showed that the development of rhizosheaths is typical of xeromorphic grasses (Price 1911; Oppenheimer 1960; Leistner 1967; Wullstein et al 1979; Wullstein and Pratt 1981 and Buckley 1982) growing on sandy soil (Price 1911; Oppenheimer 1960; Leistner 1967; Wullstein et al 1979; Wullstein and Pratt 1981; Buckley 1982; Goodchild and Myers 1987 and Marneweck 1990). Some studies have reported sheath occurrence in some mesomorphic grass species (Duell and Peacock

1985; Goodchild and Myers 1987) and in clay loam soil (Duell and Peacock 1985).

On commencing this study a pilot investigation was conducted. Pressed herbarium specimens of grasses occurring in southern Africa were briefly surveyed and it appeared that if sheaths were present on one individual of a species then all individuals of that species exhibited sheath development. In addition, if one species exhibited sheath development then all the species of that genus developed sheaths, some more distinct/obvious than others. These findings suggested that sheath presence is a genetically fixed trait, expressed in all individuals of certain genera. Whether the grass specimens were collected from regions having similar soil and/or climatic conditions was not considered and therefore, there was a possibility that these environmental conditions could affect sheath development. Marneweck (1990) showed that species which exhibited rhizosheaths when growing in sandy soils are not restricted in their distributions to sandy soil areas. However, it was not investigated whether individuals on the non-sandy soil develop rhizosheaths.

1.4 RATIONALE

Information available on rhizosheaths is limited, especially with respect to South African grass species. Many hypotheses have been proposed but little is known about their development in response to the environment or their functions.

Of the studies conducted on rhizosheaths, most were anatomical (Price 1911; Wullstein and Pratt 1981; Buckley 1982; Vermeer and McCully 1982 and Marneweck 1990). One study investigated nitrogen fixation associated with rhizosheaths (Wullstein et al 1979), another the occurrence of sheaths on mesic grasses (Duell and Peacock 1985) and another investigated xylem vessels and their functioning in relation to rhizosheath presence (McCully and Canny 1986). Only one detailed study on sheaths occurring

on grass species in South Africa (Marneweck 1990) had been undertaken, although sheaths had previously been reported on some South African grasses (Leistner 1967).

From the studies it appeared that rhizosheaths occur mainly on grasses growing on sandy soil in arid areas. This implied that sheath development is a facultative response to sandy and/or arid conditions. Whether this is a species characteristic or an individual response to the environment had never been investigated. No study had been done to determine the possible functional role(s) of rhizosheaths.

A study was undertaken to investigate rhizosheaths on South African grasses. The aim of this study was to gain information on the occurrence of sheaths among South African grass species, the influence of environmental conditions on the extent of their development, and the possible functions of rhizosheaths.

Determination of which species exhibit sheath development would give an indication of whether sheath presence may be used in Poaceae field identification keys as an easily recognisable characteristic of certain species, genera or tribes. The capacity to develop sheaths must be under some level of genetic control. If the trait is expressed in all individuals of a species then this trait may be one which would differentiate between species which might be easily confused with one another, especially when only vegetative material is available. If the sheaths are not present on all individuals of a species it would not be possible to use the characteristic in field identification keys.

Information on how rhizosheaths function to increase plant fitness/vigour would lead to an understanding of why certain grass species develop these sheaths and how these species are suited for survival on sandy soil and/or in semi-arid areas. A large percentage of South Africa is semi-arid to arid, as well as being sandy. Therefore, if rhizosheaths do increase the

vigour of grass plants and enable them to tolerate conditions of low nutrient and low water availability, and if the presence of sheaths is a genetically fixed trait, then crop plant growth in dry, sandy areas may be significantly increased, provided they could be genetically manipulated to develop rhizosheaths.

The research study was divided into three main sections. The first investigated rhizosheath distribution among grasses in South Africa. The second section considered the influence of environmental conditions on sheath development. The final section investigated the possible functions of the sheaths. The thesis has been divided into four main parts, as shown in Figure 1.3.

PART I INTRODUCTION

CHAPTER 1

- 1) What are rhizosheaths?
- 2) In which species are they known to occur?

CHAPTER 2

- 1) Literature review.

PART III POSSIBLE FUNCTIONS OF RHIZOSHEATHS

CHAPTER 5

- 1) Compensation for low water availability.
- 2) Compensation for low nutrient availability.

CHAPTER 6

- 1) Influence of rhizosheath thickness on nutrient uptake.

CHAPTER 7

- 1) Rhizosheath influence on the microbial biomass at the soil-root interface.

PART II THE DISTRIBUTION OF SHEATH FORMING SPECIES AND THE ENVIRONMENTAL INFLUENCE ON THEIR DEVELOPMENT.

CHAPTER 3

- 1) Which species exhibit rhizosheath development and the extent of the development?
- 2) The distribution of sheath forming species as affected by soil type and rainfall conditions.

CHAPTER 4

- 1) Sheath development as a facultative response to soil texture.
- 2) Sheath development as a facultative response to soil water and nutrient status.

PART IV SUMMARY AND CONCLUSIONS

CHAPTER 8

- 1) Overall perspective of rhizosheath occurrence and functioning.

Figure 1.3. Sections of the research study and layout of the thesis.

CHAPTER 2

REVIEW OF RELEVANT LITERATURE

2.1 RHIZOSHEATH PRESENCE ON SPECIES OTHER THAN THOSE IN THE FAMILY POACEAE.

Investigations into which plant species exhibit rhizosheaths have been conducted to a limited degree (Leiser 1968; Duell and Peacock 1985) and it has been found that the distinct rhizosheaths described in chapter 1 are characteristic of the Poaceae (Duell and Peacock 1985). Certain members of the Cyperaceae produce a type of rhizosheath however, it has not been investigated whether the structure and composition of this sheath are the same as those in the Poaceae.

Mucilage sheaths occur on the roots of a range of monocotyledonous and dicotyledonous plant species. These include ericas (Leiser 1968), certain citrus species (Brams 1969), radish (Dawes and Bowler 1959), maize (Leech, Mollenhauer and Whaley 1963; McCully and Canny 1986), wheat (Northcote and Pickett-Heaps 1966; Pickett-Heaps 1967 and Buckley 1982), and oats (Namibiari 1976). In most cases the presence of a mucilage layer does not result in the formation of a distinct, hardy sheath of sand grains as described in chapter 1. The precise function of the mucilage sheath is not yet known and the cross sectional structure of the sheaths and roots has not been investigated. However, similar functions to those proposed for the sand grain sheaths have been proposed for these sheaths, because of the resulting intimate proximity of soil particles to the root due to binding by the mucilage. It has also been hypothesised that the sheaths may increase root growth in some way, since roots with the most well developed sheaths appeared to be the most vigorous, rapidly growing roots (Leiser 1968). According to

Leiser (1968) these sheaths may be used to assess the status of root growth in the family Ericaceae.

2.2 THE FORMATION AND MORPHOLOGY OF GRASS RHIZOSHEATHS.

Rhizosheaths develop as the roots grow through the soil and probably begin developing as the primary root begins to grow down through the soil. It has been proposed that rhizosheath development is a response by perennial grass species to dry, sandy soil conditions (Leistner 1967).

The tip of the root is covered by a root cap, which is a mass of cells that protects the apical meristem, and through the secretion of a mucilage layer, aids the root in its penetration of the soil. As the root grows the root cap is pushed forward and the cells on the periphery of the cap become detached. peripheral tissue over the entire cap synthesises and releases mucilage into the adjacent soil (Guinel and McCully 1987). The secreted mucilage and detached cells, which carry with them some root cap mucilage, are left behind by the growing root. As the mucilage comes into contact with the soil, sand and silica particles adhere to the mucilage and become embedded in it. The mucilage carries numerous carboxyl groups, and therefore negative charges (Miki, Clarke and McCully 1980), which are able to bind to positively charged minerals and soil particles (Jenny and Grossenbacher 1963). The mucilage secreted by the root cap, disintegrating cortical cells and the detached living cells, as well as the dead detached cells are involved in soil binding and formation of a sand grain sheath around the length of the root. As the root expands and an increasing number of epidermal hairs are produced so more particles become embedded in the mucilage and are bound together with the aid of the epidermal hairs (Wullstein and Pratt 1981). In sheath forming species the epidermal hairs are often exceptionally long relative to those on non-sheath forming species (Marneweck 1990). The epidermal hairs may not only be responsible for mechanical binding of the

sheath, since in certain species, for example *Stipagrostis uniplumis* var *uniplumis*, some of the hairs have been shown to exhibit terminal swellings which are probably vesicles which secrete mucilage that binds the sheath particles together (Marneweck 1990).

The extent to which the sheaths develop, i.e. the thickness of the sheath around the root and the degree to which the sand particles are bound to the sheath, varies within and between species. It has been found that different grass species exhibit different ratios of root hairs to mucilage and the size of the sheaths depend on the plant species size (Duell and Peacock 1985). An ability to increase the production of root hairs often corresponds to the ability to colonize arid habitats (Kutschera 1960 in Leistner 1967). The more root hairs present the greater the number of soil particles which may be held and the tighter they may be held. The tighter the particles are held, the more consolidated the sheath, the greater the degree of soil stabilization around the plant roots.

An important phenomenon in grasslands is the high below-ground productivity (section 2.3). This may be as a result of the relationship between the grass roots and the soil biota which is unique to rhizosheath forming grass species due to rhizosphere modifications by sheath development (section 2.4).

2.3 GRASSLANDS AND THEIR BELOW-GROUND PRODUCTIVITY

Compared to other terrestrial ecosystems, grasslands are structurally simple and appear to be relatively homogeneous. However, they are rich, functionally complex systems, particularly the below-ground component, which is often underestimated. Sixty to ninety percent of the net primary productivity in grasslands often occurs as roots and often ninety percent of secondary productivity occurs below-ground as soil microorganisms (Coleman, Andrews, Ellis and Singh 1976; Stanton

1988).

In the below-ground system there are at least two trophic pathways: herbivory and decomposition. The pathways are regulated by the primary resource, roots, as well as by many species of secondary decomposers. All growing roots provide a diverse chemical and morphological array of pulsed and temporarily varying resources for consumption by microorganisms, and are the preferred foraging sites for a variety of soil microorganisms. Roots, particularly in the region of the root cap, are surrounded by a mucigel, which consists of substances secreted by the root cap tissue and by the microorganisms which rapidly colonise the root secreted mucilage.

Rhizosheaths may contribute substantially to the below-ground productivity in grasslands. This would however depend on how common these sheaths are among grass species and how they affect grass root functioning as well as the adjacent rhizosphere microorganism populations and their activities.

2.4 THE RHIZOSPHERE

The soil zone immediately adjacent to the roots is different from the bulk soil in terms of microbial populations, chemical properties, and physical properties (as in the case of rhizosheaths). This zone is termed the rhizosphere and since it is a physically and chemically variable zone its boundaries are ill-defined. The rhizosphere environment is influenced by processes such as root uptake of water and nutrients and the release of products from the roots (Rovira, Foster and Martin 1979) as well as from detached living root cells (Guinel and McCully 1987) and alteration of oxygen-carbon dioxide levels. Many of the released products stimulate growth of microbial populations which in turn affect the rhizosphere environment through the release of products, such as plant growth hormones and mucilage, as well as through microbial mineralization and

fixation of mineral nutrients.

Although the chemical properties of the bulk soil, such as pH, are important for root growth and mineral nutrient availability, the conditions in the rhizosphere and the degree to which roots can modify them, ultimately determine mineral nutrient uptake (Marschner 1986). Roots alter the pH of the rhizosphere through the release of carbon dioxide, hydrogen ions and hydrogen carbonate ions, or through a greater uptake of water than ions. The solubility, and hence availability, of certain nutrients is regulated by soil pH. For example, the amount of available phosphorus in the soil increases as the rhizosphere soil becomes more acidic (Boero and Thien 1979).

The rhizosphere does not represent a fixed distance from the root surface but is thought to be a continuum of activity from a maximum at the root surface, decreasing gradually to a point of no influence in the soil matrix (Wollum 1982). Generally it extends from the root surface to between 1mm and 10mm from the surface (Jenny and Grossenbacher 1963). It is often divided into three regions: the rhizoplane, which is the root surface; the inner rhizosphere, which is generally considered to extend 1.5mm - 2.0mm from the root surface; and the outer rhizosphere, which can be as much as 10mm from the root surface.

The description of rhizosheaths as modifications of the rhizosphere (Atlas and Bartha 1987) was indeed accurate since sheaths include the root surface and may extend up to 3mm from the surface, and therefore include at least the inner rhizosphere.

2.5 SOIL TEXTURE: SANDY VS CLAY SOIL.

Although some soils are nearly 100% sand or 100% clay these are rare. Usually soils are a combination of sand (particles 2.0-0.05mm in diameter), silt (particles 0.05-0.002mm in diameter) and clay (particles less than 0.002mm in diameter), the relative proportions of which dictate the texture of the soil. A combination of sand, silt and clay would be the most favourable soil environment for plant growth since each has properties which are favourable and some which are unfavourable for plant growth. Each soil type provides specific conditions (for example water availability, nutrient status and soil texture) for root growth. Therefore, sheath development may be a facultative response to one or more of these conditions in the soil.

Sandy soil has a low bulk density (dry mass per unit bulk volume) whereas clay soil has a high bulk density. Sandy soil has a low surface tension and a high infiltration rate and therefore, drains fast and holds little water relative to clay soil, which has a high surface tension. In semi-arid and arid regions the rapid draining creates a problem for plant growth since the soil is not frequently rewetted, and is therefore mostly dry.

Unfavourable characteristics of clay soils include the small inter-particle spaces (pores), and this may impede root penetration through the soil because roots only grow unrestrictedly in pores of diameters greater than their own diameter (Greenland 1979). Another characteristic of certain clay soils which impedes plant growth is the high swell-shrink capacity, due to the presence of swelling clay minerals such as smectites. This phenomenon causes the soil to swell when wet and shrink and crack when dry and in so doing disturb the roots. Soil as a whole has no net charge, but at a molecular scale, the distribution of charges is uneven. A soil patch with a net negative charge is said to have a cation exchange capacity (CEC), and one with a net positive charge, an anion exchange capacity (AEC). In moist soils the CEC is much greater than the AEC. The

CEC is a measure of the soils ability to hold nutrients against leaching. The CEC, when measured at soil pH and ionic strength, may be used as an indication of the soil fertility. Sandy soil has a low CEC and therefore, has a limited ability to hold nutrient cations against leaching. This, together with the fact that water drains rapidly through sandy soil, means that the time available for roots to take nutrients up from the soil is short relative to the time available in clay soil. Clay is generally more fertile than sandy soil because it has a high CEC and therefore holds nutrients against leaching, thereby allowing the roots a longer period for nutrient uptake.

2.6 PLANT ROOTS AND THEIR INTERACTION WITH THE SOIL.

Plant roots have a number of functions. These functions include anchoring terrestrial plants in the soil; acting as food storage organs and they are the site of synthesis of a number of plant hormones. The function most relevant to this study is the absorption of water and nutrients from the soil which is followed by their transport to the above-ground plant parts.

Absorption and transport of nutrient ions and water by the roots of intact plants are complex processes. These processes depend not only on the properties of the cell membranes, but also on the structural organization of the root as it ages, the requirements of the plant, as well as the numerous soil factors which continuously influence the growth and function of the root system.

The main soil factors which influence the growth and function of roots in soil are the soil water potential, oxygen concentration, mechanical resistance, and the concentration of nutrient ions and other solutes. For each factor the range favourable for root metabolism is narrow, so that roots are almost always subject to some degree of stress (Drew 1979).

2.7 SOIL WATER AND ITS UPTAKE BY PLANT ROOTS.

Water can be held by the soil either in the pores between the soil particles or by adsorption on the surfaces of the clay and organic matter particles (Childs 1969).

The intensity with which water is held by a soil can be measured in units based on the concept of suction (Russell 1973). Soil water suction increases as the water content of the soil decreases, therefore, the suction force required by the roots to extract water from the soil must increase correspondingly. The soil will deliver water to the roots provided the suction exerted by the roots is greater than the soil suction (Hillel 1971).

Water in a sandy soil, which has recently been wetted, is held at a low suction level and only the last 6-7% of soil water is held at a high suction level (Russell 1973). Water drains rapidly through sandy soil. Therefore, in semi-arid and arid areas, where the soil water is not frequently replenished, soil water suction in sandy soils must generally be at high levels. In hot, dry areas this is further exacerbated by the high soil temperatures. Soil temperature affects the rate of water movement through the soil under given suction gradients due to the effect on its viscosity. Increasing soil temperatures increase the speed at which water can leak out of the soil profile. This leak is often large enough to cause the suction of residual water to rise despite the fact that an increase in temperature would have lowered the suction of water if the water content had remained constant (Russell 1973). Consequently, plants growing on these soils must generally exert high suction pressures at the soil-root interface in order to absorb water.

In clay soil, water drains more gradually and therefore suction increases gradually. At any particular root suction pressure, clay soil retains more moisture than a sandy soil (Hillel 1971). In the unsaturated state a clay soil has a higher water content and hydraulic conductivity than a sandy soil and plants growing

on this soil can therefore maintain a high transpiration rate for longer (Hillel 1971) than those on unsaturated sandy soil.

The rate of water uptake from a given volume of water depends on rooting density, soil conductivity and the difference between the average soil water suction and root suction (Hillel 1971). Water absorption takes place directly through the epidermis of the root. The epidermal root hairs provide an enormous area for absorption. More than 90% of the water absorbed by the roots is lost from the leaves to the atmosphere as water vapour, through the process of transpiration. As water is lost from the leaves so more water moves into the leaves and more water must move into the roots and up the plant, through the xylem, to the leaves. A transpiration stream through the plant is created. During periods of rapid transpiration, water may be removed from around the roots so fast that the soil becomes depleted. Water will then move from some distance away towards the root hairs through the soil pores.

2.8 SOIL NUTRIENTS AND THEIR UPTAKE BY PLANT ROOTS.

The availability of nutrient ions to plants depends on their mobility and proximity to adsorbing surfaces. Dissociated ions in the soil solution and the majority of exchangeable ions adsorbed onto colloidal surfaces are generally readily available for root uptake. Non-exchangeable ions in primary minerals and organic matter are usually not readily available (Richards 1987). The mobility of ions is determined principally by mass flow and diffusion, the two processes which govern the rate at which solutes move from the bulk soil towards uptake sites on the root surface. The rate of mass flow of a solute is proportional to the volume of water flow and the concentration of the solute in the soil water (Singer and Munns 1987). Diffusion is the movement of individual ions, even without water movement, when differences in ion concentration develop in the soil (Singer and Munns 1987). The rate of diffusion of a solute depends on, among

other factors, the magnitude of the concentration gradient (driving force) and the ion's diffusion coefficient (ease of movement) in the soil (Singer and Munns 1987). Nutrient elements such as nitrogen and phosphorus are often so dilute in the soil solution that mass flow of soil water to meet transpiration losses provides only a small percent of the total plant requirement. Consequently, the bulk of these elements moves to the root surface by diffusion (Prenzel 1979; Chapin 1980). Highly mobile cations, such as calcium and magnesium, move readily to the root surface by both mass flow and diffusion and often accumulate around the root when supply exceeds absorption (Nye 1977).

The rate of uptake of nutrients by the root system depends partly on the rate that they are brought from the bulk soil to the root surface, which is predominantly dependent on soil factors, and also on the rate at which they can be transferred from the root surface into the root. Some ions (usually anions) are taken up by active transport processes, using membrane carrier proteins, while others (cations) move into the root passively, for example by ion exchange, or by diffusion due to the water potential across the plasma membrane that is created by the ions which are moving in actively. The relative significance of active and passive uptake mechanisms varies with soil conditions and plant species (Richards 1987). The rate at which nutrient ions are transferred from the root surface into the root is in turn predominantly dependent on plant factors such as size of the root system, growth rate and demand of the above-ground plant parts for the nutrients (Russell 1973).

Nutrient ions may either be mobile or immobile. Ions showing mobile behaviour are those which are supplied to the root by mass flow alone, in sufficient quantities to meet the plants requirements. Ions show immobile behaviour if rhizosphere depletion makes diffusion an essential supply mechanism (Singer and Munns 1987), every nutrient ion species becomes immobile when it is deficient. Some nutrients, such as phosphorus, show

immobile behaviour even under normal, nondeficient conditions.

In fertile soil the rate of mobile nutrient ion uptake, especially nitrate (NO_3^-), is often limited by the rate at which the ions move from the soil into the root (Russell 1973). The rate of uptake in less fertile soil (for example sandy relative to clayey soil), and uptake of immobile ions, is limited by the rate at which the ions are transported to the root (Russell 1973).

If a solute is absorbed faster than water, as happens with phosphate, then its concentration in the soil solution at the root surface will fall. This change is buffered by the release of ions adsorbed onto soil particles. If however, water is absorbed faster than the solute then the solute collects at the root surface and will then tend to diffuse away from the root.

2.8.1 Nitrogen: its uptake by roots and functional role in plants.

Most of the nitrogen (N) in soils is derived from the atmosphere. Atmospheric nitrogen is converted to a plant available form by certain microorganisms living in the soil. This process is termed "nitrogen fixation". Once fixed, N is recycled through the system by a complex array of processes and the functioning of many natural ecosystems depends on the bacterial fixation of atmospheric N (Atlas and Bartha 1987).

The N content in soils varies widely and is an important factor in determining the geographical distribution of plant species (Bradshaw, Chadwick, Jowett and Snaydon 1964). High soil N concentrations are often considered indicative of high nutrient status soils. Similarly, the total N concentration in plant tissue is indicative of the nutritional value of the plant.

Approximately 99% of all N in terrestrial ecosystems is organically bound and thus an accumulation of soil N closely follows that of soil organic matter. Organic N is mineralized into plant available forms, for example ammonium (NH_4^+), by microbial processes (Anderson and Ingram 1993). Organic matter binds to clay particles and therefore soils rich in clay have a higher organic matter content than loamy and sandy soils, except when clay minerals occlude organic matter.

Nitrogen is an essential macronutrient, required for the growth of all plant species. It can be taken up by the roots in the form of nitrate (NO_3^-) or ammonium (NH_4^+). Nitrate moves into the root by active transport mechanisms. The transport system releases a hydroxyl ion (OH^-) to the soil from the root while simultaneously moving a NO_3^- ion into the root (Haynes 1990). Ammonium (NH_4^+) has only been observed to move passively into the root. Nitrogen is essential for amino-acid synthesis and therefore the production of proteins as well as production of nucleotides, nucleic acids, coenzymes and chlorophyll. The bulk

of leaf N is directly involved in photosynthesis as a component of photosynthetic enzymes and chlorophyll (Chapin 1980). Thus, over a broad range, photosynthetic rate is proportional to leaf N concentration.

The most common visual signs of N deficiency in plants is chlorosis of the leaves and an etiolated habit (Epstein 1972). The older plant parts are affected first because, when growing in conditions of low N availability, plants may translocate N from these leaves into the developing plant parts (Marschner 1986).

2.8.2 Phosphorus: its uptake by roots and functional role in plants.

Phosphorus (P) in the soil is derived from the parent material. While P is not an abundant element in the soil its availability is further restricted by its tendency to precipitate in the presence of divalent metals (Ca^{2+} , Mg^{2+}) and ferric (Fe^{3+}) ions, at neutral to alkaline pH (Atlas and Bartha 1987). This precipitation renders P unavailable for plant uptake.

The immediate source of phosphate for plant uptake is probably that of the inorganic phosphate ions in the soil solution, the concentration of which varies widely for different soils (Russell 1973). Organic phosphorus is also present in the soil solution and in soil organic matter. P is cycled through the system via a number of transfers (Atlas and Bartha 1987). Microorganisms are essential mediators of such transfers, for example between insoluble, immobile forms and soluble, mobile forms, or inorganic and organic phosphate.

Phosphorus is an essential macronutrient, required for the growth of all plant species, although it is needed in much smaller quantities than nitrogen. It is taken up by plant roots in the form of H_2PO_4^- and $\text{H}_2\text{PO}_4^{2-}$, depending on the soil pH. The

availability of both these forms varies with changes in the soil pH. Since both forms of phosphate are anions they are probably taken up by active uptake mechanisms. Phosphorus moves to the root surface primarily by diffusion, but also by mass flow. The P depletion zone around the root is much narrower than that of ammonium N (Jianguo and Shuman 1991).

Phosphorus is required for the synthesis of adenosine tri- (and di-) phosphate (ATP and ADP), essential for the transfer of energy within cells. In addition, it is required for the formation of phospholipids, nucleic acids, coenzymes and the phosphorylation of sugars.

The uptake of P is affected by a number of factors. These include the presence of other ions in the soil, for example the presence of ammonium ions may assist P uptake since their presence would increase the soil pH thereby altering the availability of P. The rate of P uptake also depends on the soil temperature, being lower in cold weather (Russell 1973). Root exudates and the presence of products of microbial metabolism also affect P uptake since they may function to increase P solubilization, due to the organic anions present in these products which have been shown to be effective in reducing P adsorption to clay mineral surfaces (Bhat, Nye and Baldwin 1976). Another factor influencing the rate of P uptake is the concentration of P close to the root surface and the rate at which the extracted P can be replenished by diffusion from the bulk soil (Russell 1973). The rate of P uptake is limited by the rate at which it is brought to the root surface rather than the rate of its transfer into the root. At times, in P deficient soils, insufficient P is brought to the root surfaces to meet the plant requirements. Under these conditions, plants benefit from the increased exposure to P provided by a rapidly developing and finely divided root system (Gerloff and Gabelman 1983).

The presence of microorganisms, especially mycorrhizal fungi (Kothari, Marschner and Romheld 1990), on plant roots has been

shown (Bowen and Royira 1966 in Richards 1987; Barber 1969) to greatly modify the absorption and utilization of phosphate by roots, especially when the supply of phosphate is low. Plant roots infected with vesicular-arbuscular mycorrhizal fungi have a higher phosphorus absorption capacity compared to non-mycorrhizal roots (Kothari et al 1990). Mycorrhizal hyphae are able to absorb and translocate P from distant areas which otherwise are not accessible to plant roots (Rhodes and Gerdemann 1975). It has been shown that associations between plant roots and mycorrhizal fungi influence nutrient uptake by roots. This is particularly true for P uptake.

Common signs of P deficiency in plants is the development of purple, red or brown pigments in the leaves, especially along the veins. Growth may be reduced and under severe deficiency the plants become stunted (Epstein 1972).

2.9 POSSIBLE FUNCTIONS OF RHIZOSHEATHS.

The occurrence of rhizosheaths was reported as early as 1887 by Volkens (Wullstein et al 1979). Since then some research has been carried out on rhizosheaths, but most of the work has been concerned with their structure. Many possible functions have been proposed by those who have studied sheaths, but few of these have been fully investigated. Below is a list of the functions which rhizosheaths may perform.

- i) The sheaths may enhance water retention at the root surface (Price 1911; Wullstein et al 1979) and protect root hairs from desiccation in hot, arid environments (Price 1911; Marneweck 1990) since roots with mucilage sheaths plasmolyse much slower than roots without sheaths (Leiser 1968).
- ii) They may, together with the hyperdermis, insulate the stele against moisture loss (Leistner 1967; Clarkson and Hanson

1980 and Buckley 1982).

- iii) The sheaths may form a physical barrier which offers protection to the root against: a) organisms and their enzymes (Oades 1978); and b) mechanical abrasion and soil compression (Thomas 1922; Oppenheimer 1960), especially on roots with thick, well consolidated sheaths.
- iv) They may promote water absorption in arid conditions (Price 1911; Henrici 1929 and Marneweck 1990).
- v) The sheaths may reduce moisture loss in roots during water translocation from roots in damp soil to plant parts in dry soil (Buckley 1982).
- vi) Due to the increased production of root hairs the sheaths may increase water and nutrient uptake (Goodchild and Myers 1987).
- vii) Rhizosheaths may increase nitrogen fixation rates immediately adjacent to the root surface (Wullstein et al 1979).

These hypotheses have not been adequately tested, except perhaps the one concerning microbial nitrogen fixation, since nitrogen fixation has been shown to be associated with rhizosheaths on certain grasses (Wullstein et al 1979). However, the uptake of fixed N was not investigated.

All plant species differ in their response to nutrient stress. Detailed research has been done on responses such as modification of the root/shoot ratio (Whiteaker, Gerloff, Gabelman and Lindgren 1976; Chapin 1980), modification of root division (Hackett 1969), modification of ion uptake mechanisms (Lauchli 1976) and increased mycorrhizal associations (Mosse 1973; Chapin 1980 and Kothari et al 1990). However, no investigation of rhizosheaths as a response to nutrient stress has been

undertaken.

Different plant species show different response mechanisms to nutrient stress. Some will divert an increased proportion of plant reserves into root production at the expense of shoot production (either into more lateral roots (Hackett 1969) and/or root hair growth (Barber 1973) or into extremely long tap roots) when growing under stressful conditions (Ulrich and Berry 1961 and Schenk and Barber 1979 in Gerloff and Gabelman 1983). This creates a greater area of root surface that can come into contact with any available nutrient ions (Whiteaker et al 1976).

There is a low degree of branching (Price 1911; Goodchild and Myers 1987) in root systems which exhibit extensive sheath development. This suggests that the development of rhizosheaths may be a substitute for increased lateral root production due to factors which will be discussed in sections 2.9.2 and 2.9.3.

2.9.1 Protection of roots and root hairs.

Due to its pectic constituents the sheath mucilage binds readily to cations and may thus serve to protect the roots against penetration of heavy metal cations towards the apical meristem prior to the development of the Casparian strip (Barlow 1975).

Similarly, the sand grain sheath may offer a physical barrier to allelochemicals produced by neighbouring plants and/or organisms which may otherwise adversely affect the plant through enzyme synthesis and penetration of the roots.

Price (1911) hypothesised that the sand-mucilage sheath serves to prevent desiccation of root hairs which, under conditions of extreme heat and drought, would shrivel as they extend through the soil if unprotected. As the soil dries the roots may shrink slightly, the layer of soil around the root would be drawn closer to the root. This layer contains water-filled pores which form a protective buffer against further desiccation and help preserve

a moisture film around the root (Greenland 1979). Since the sheath mucilage has a high affinity for water absorption (Price 1911) it may be an important method of conserving water for the roots. This hypothesis is supported by the fact that Wullstein et al (1979) found a higher moisture content (on a dry weight basis) in the sheath (12-25%) of certain xeromorphic grass species than in the surrounding bulk soil (3-8%). This high moisture content would also promote microbial activity (Wullstein et al 1979).

2.9.2 Water and nutrient uptake

Price (1911) reported the presence of the rhizosheaths on a number of perennial grasses which are indigenous in North Africa. From the results of his anatomical work on rhizosheaths he hypothesised that the intimate relationship between sand, mucilage and root hair promotes water absorption in the arid, permeable surface soil of the sandy desert areas in which the species he studied grew.

A close contact between roots and sufficient soil material can lead to solubilization of enough micronutrients in the mucilage for the plant's requirements (Marschner 1986). In support of this, there is evidence that in dry soils there may be an increased production of mucilage by the roots (Drew 1978). The mucilage may also have a role as ion exchanger between soil and root and is probably of value to the plant for nutrient uptake from dry soils. Nutrient ions dissolve in the sheath mucilage (Leiser 1968) and thus become more readily available for uptake by the root.

The development of prolific epidermal hairs, which are often of substantial length (Marneweck 1990), and which correspond to sheath development, may significantly increase the uptake capacity of roots growing under conditions of low water/nutrient availability (Buckley 1982). In addition, the close proximity of the prolific epidermal hairs, soil particles, water, mucilage

and dissolved nutrients may facilitate easier absorption of water and nutrients by the root system. In addition, sheaths may also provide an increased supply of nitrogen due to increased nitrogen fixation associated with the sheaths (Wullstein et al 1979).

These factors would reduce the need for sheathed plants growing in conditions of low nutrient and water availability, to channel increased proportions of their reserves into root production in order to increase the root absorptive surface area.

2.9.3 Microbial activity.

All plant roots influence the rhizosphere, which is an area of intense microbial activity since it is an environment which stimulates bacterial and fungal growth. The microbial biomass is normally about ten times higher in the rhizosphere than in the bulk soil (Singer and Munns 1987). The numbers of microorganisms, the most abundant of which are bacteria (10^9 per gram soil), in the rhizosphere can be up to 100 times greater than in the bulk soil (Katznelson 1965; Gray and Parkinson 1968).

There is a positive feedback relationship between rhizosphere processes and the roots. The interactions of plants and microorganisms are based largely on interactive modifications of the soil environment by processes such as root uptake of water and nutrients, the release of organic products (exudates, secretions, lysates and gases) to the soil by roots, as well as the microbial production of plant growth factors and microbial mediated availability of mineral nutrients.

Products released from the root may inhibit (through toxicity to certain microorganisms) or stimulate (provide energy for microbial activity) growth of certain microbial populations. Plant roots therefore have a direct effect on the composition and diversity of the rhizosphere microbial community. On the other hand, in the absence of appropriate microbial populations, plant growth may be impaired (Lynch 1976; Dommergues and Krupa 1978 and

Campbell 1985).

The ways in which microbial populations may benefit plants include the synthesis of vitamins, amino acids, auxins and gibberellins, which stimulate plant growth. The microorganisms also influence the availability of mineral nutrients to plants. For example, the microbial biomass is an important regulator of labile nitrogen in the soil (Campbell 1976 in Doran 1980), since the microorganisms carry out N mineralization and immobilization (section 2.10).

It may be speculated that the rhizosheaths provide an even more suitable rhizosphere environment for microbial activity than an unaltered rhizosphere. This more favourable environment closer to the root surface may be a result of the higher water content in the sheath relative to the adjacent soil (Price 1911).

There is an ongoing debate as to whether it is beneficial or detrimental to the plant to have a high concentration of microorganisms in close proximity to the roots. There is evidence that the rhizosphere organisms can help mobilize plant nutrients, such as iron, zinc and copper (Singer and Munns 1987) and nitrogen, through increased recycling and solubilization. Nitrogen is often a limiting factor in soils and free-living nitrogen fixing bacteria perform a vital function for certain plant species by providing their roots with nitrogen. Wullstein et al (1979) showed that in addition to the sheath serving as a repository for moisture, it is a sink for organic energy sources required for nitrogen fixation, which they proved was associated with the sheaths of the grasses they studied. These researchers concluded that the rates of fixation in the sheaths may be significant to the nitrogen economy of these grasses.

On the other hand, microorganisms may, through the utilization of the soil nutrients for metabolism, immobilize limiting concentrations of the nutrients and render them unavailable to the plant (Nicholas 1965; Barber 1978). In addition, many

microorganisms are pathogenic and thus detrimental to the plant. Other harmful microorganisms include those which can alter the permeability of root cells, the presence of these organisms would increase the organic product loss from the roots (Rovira and McDougall 1967; Bowen and Rovira 1976). This rate of loss of organic substrates from the roots to the soil may be doubled by the presence of microorganisms at the root surface (Anderson and Ingram 1993). If the losses become too great they may retard plant growth. However, the effect of such losses are probably outweighed by the benefits accrued to the plant by the development of an efficient nutrient turn over by the microorganism population in the rhizosheath, particularly if the plant is limited by environmental or nutritional factors rather than carbon.

An interesting phenomenon which occurs in certain rhizosphere bacteria is the production of sticky coatings which help them adhere to one another, root cells and soil particles (Singer and Munns 1987). Certain microorganisms, which have been isolated from rhizosheaths, produce mucilage (Wullstein et al 1979), this together with the mucilage secreted by the roots is referred to as mucigel (Jenny and Grossenbacher 1963; Rovira et al 1979). Since the populations of bacteria may be in higher concentrations in the rhizosheaths than in the rhizosphere of unsheathed roots, they may be partly responsible for the formation of the sheath. The mucilage secreted by the grass root and the epidermal hairs may initiate sheath development and the conditions created therein would promote microbial colonization. Some microorganisms exhibit positive chemotaxis to root exudates, which enables them to move rapidly to the roots (Rovira 1965) and colonize the rhizosheath. The colonizing mucilage-secreting bacteria would consequently assist in further consolidation of the sheath. However, because there is a high degree of spatial heterogeneity of bacterial distribution in the soil the root is not uniformly in contact with bacteria (Anderson and Ingram 1993). Since sheaths are of uniform thickness and extend the entire length of the roots it would therefore seem that products

released from the roots are more important than microbial mucilage in sheath formation. However, the importance of the cementing effect of microbial mucilage must not be underestimated. It was found (Wullstein and Pratt 1981) that sheath sand grains began separating towards the end of summer, when the size of the active microbial populations probably decreased. This indicates that due to a decrease in microorganisms there was a decrease in cementing agents between sand grain interfaces further from the root at distances where the root mucilage was less abundant and therefore less effective in binding sand grains.

2.10 THE ROLE OF MICROORGANISMS IN NUTRIENT CYCLING.

Availability of nutrients markedly influences plant vigour and consequently productivity. Therefore, microbial processes affecting nutrient availability have a vital role in ecosystem functioning. Although microbes may adversely affect plant growth, by competing for nutrients, they generally affect plants favourably, by making nutrients available for root uptake. Nutrients become available as by-products of the microbial respiration of organic substrates (Richards 1987). When inorganic ions are produced by the oxidation of organic compounds the process is termed mineralization. When inorganic molecules are assimilated into microbial protoplasm the process is termed immobilization. Microbial mineralization and immobilization occur concurrently such that there is a continual biological turnover in the soil. The energy required to maintain this mineralization-immobilization cycle is that released during the oxidation of organic compounds.

2.10.1 Nitrogen, carbon and phosphorus .

Ammonia is a waste product of microbial metabolism and any ammonium N that accumulates in the soil represents the quantity of substrate N in excess of microbial requirement. Organic matter added to the soil normally contains both C and N, the relative proportions of which determines whether ammonium N will accumulate in the soil. Generally, net mineralization occurs in a system where the C:N ratio is 20:1 or less, while net immobilization for a period of weeks or months may occur in a system where the organic matter has a C:N ratio of 30:1 or greater (Richards 1987).

Plants are an important source of C for many ecosystems. The breakdown of C and the recycling of this element is dependent on microbial activity. Within the soil, C is present in different forms and is not always readily available for microbial utilization. The soluble, readily available soil C occurs generally within the rhizosphere as a result of both root release of primary products and microbial activity. This C is recycled relatively rapidly and some is released to the environment as carbon dioxide. Carbon may also be present in humic form which is broken down by microbes at a much slower rate than soluble C. Carbon that is incorporated in 2:1 clay layers of silicon and aluminium is almost unavailable to soil microbes and this stable C pool turns over extremely slowly.

While much of the phosphate taken up by plants is provided by the weathering of primary materials, the microbial oxidation of organic substrates is an important supplementary source of inorganic phosphate. Phospholipids, nucleic acids and sugar phosphates undergo rapid degradation to inorganic phosphate, but the mineralization of other phosphates (inositol polyphosphates) is relatively slow (Richards 1987). Small actively cycled P reservoirs occur as dissolved phosphate in soil and water and phosphate in organic matter. The polyphosphates are however an important source of phosphate for plants. Mineralization by rhizospheric microbes may not lead to increased phosphate uptake

by roots, especially in soils with low phosphate levels, where rhizosphere microbes may accumulate phosphate to the detriment of plant roots (Barber and Loughman 1970). This is a situation of net P immobilization. As for N, inorganic P derived from organic substrates will only be available for root uptake when there is net mineralization.

PART II THE DISTRIBUTION OF RHIZOSHEATH FORMING GRASS SPECIES AND THE ENVIRONMENTAL INFLUENCE ON THEIR DEVELOPMENT

Little is known about which grass species develop rhizosheaths since only a minute percentage of the world's grass species have been recorded to exhibit rhizosheath development. A limited amount of research has been done on the phenomenon of rhizosheaths, especially with respect to South African grasses. It is not known for certain under what environmental conditions the sheaths develop, although they have been recorded most frequently in sandy soil individuals and in arid conditions.

Chapter 3 will discuss which South African grass species exhibit rhizosheaths and in what soil type and rainfall conditions they most commonly occur in South Africa. The influence that these environmental conditions have on the development of rhizosheaths will be dealt with in chapter 4.

CHAPTER 3

THE OCCURRENCE OF RHIZOSHEATHS AMONG SOUTH AFRICAN GRASSES, THE DISTRIBUTION OF SHEATH FORMING SPECIES, AND THE INFLUENCE OF RAINFALL AND SOIL TEXTURE ON THE EXTENT OF RHIZOSHEATH DEVELOPMENT.

3.1 INTRODUCTION.

The first detailed study of rhizosheaths was undertaken by Price (1911). He studied the root and sheath anatomy of a few North African grass species, collected from arid sandy areas. Two of these species, *Bromus tectorum* and *Hordeum murinum*, have become naturalized in South Africa and develop sheaths.

Little is known about which species develop sheaths and whether the phenomenon of sheath development is restricted to perennial species, or whether it is more prevalent in certain tribes, genera or species. A limited amount of research has been done on rhizosheaths which occur on grass species in South Africa. Leistner (1967) recorded the presence of "sand coats" on the roots of five species (which encompassed only four tribes): *Anthephora argentea* (in the tribe Paniceae); *Aristida stipitata* (in the tribe Aristideae); *Eragrostis lehmanniana*, *Pogonarthria squarrosa* (in the tribe Chlorideae); and *Lolium multiflorum* (in the tribe Poeae). Leistner (1967) also stated that all perennial grass species growing in the southern Kalahari exhibited the sheaths, while they were absent in almost all annual species. Leistner (1967) did not however, investigate the rhizosheaths much further.

Only recently has detailed work on rhizosheaths of grasses occurring in South Africa been done. This detailed work (Marneweck 1990) involved morphological and anatomical studies of the sheaths of seven species (which encompass only three

different tribes): *Antheophora pubescens*; *Digitaria eriantha* (in the tribe Paniceae); *Eragrostis aristita*; *E. lehmanniana*; *E. pallens*; *Sporobolus rangei* (in the tribe Chlorideae); and *Stipagrostis uniplumis* var *uniplumis* (in the tribe Aristideae). Although all the specimens used in this study were collected from sandy soils, distinct variation in the anatomical structure between the species was found, especially with respect to number and structure of epidermal hairs. Marneweck (1990) stated that the modifications and structural features of the sheath forming roots which these species exhibited could be related to water stress and soil properties.

An important fact highlighted by the study (Marneweck 1990) was that although the specimens studied were all collected from sandy soil, the species distribution ranges were not restricted to sandy soil areas. A detailed investigation and study of the distribution of sheath forming individuals was not however undertaken.

Since most of the studies conducted on rhizosheaths have looked at specimens collected from sandy soil areas and generally from dry regions, it appeared that rhizosheaths are more prevalent in xeromorphic species growing in sandy soil areas. However, the distribution of sheath forming individuals in relation to climatic regions and soil types has never been adequately investigated.

Apart from a brief study (Leistner 1967) on the thickness of rhizosheaths in relation to rainfall conditions, no studies on the influence of environmental conditions on sheath development, and the extent (thickness and consolidation) thereof, have been recorded. Similarly, although the function(s) of rhizosheaths have been hypothesised (section 2.9) these have also not been adequately investigated.

As a result of this lack of knowledge two fundamental questions were addressed in this part of the study.

- i) Which South African grass species exhibit rhizosheath development?
- ii) Is rhizosheath development a genetically fixed trait or is it a facultative response to environmental conditions?

3.2 HYPOTHESIS

Following on from the second question, it was hypothesised that the development of rhizosheaths is a facultative response to environmental conditions and that individuals of a species would only develop sheaths when growing in low rainfall and/or sandy soil conditions.

3.3 MATERIALS AND METHODS

A detailed study of pressed specimens was conducted at the National Herbarium in Pretoria. Due to their hardy nature sheaths are not easily removed from the roots when the plant is extracted from the soil nor when the roots are shaken or lightly washed. They also press well.

A total of 130 species from fifteen different Poaceae tribes was studied. These tribes are Andropogoneae, Aristideae, Arundinelleae, Aveneae, Brachypodieae, Bromeae, Chlorideae, Ehrharteae, Meliceae, Oryzeae, Paniceae, Pappophoreae, Poeae, Stipeae and Triticeae. Six tribes which are represented in South Africa were not studied due to insufficient numbers of herbarium specimens of their representative species. Where possible, five genera per tribe were studied. The species were chosen on the basis of their distributions within South Africa. Those with wide distributions were preferably selected and whenever

possible, at least five species per genus were studied. All specimens which had recordings of the soil type in which they were growing at the time of collection were used. A total of 1620 specimens was studied, more than half of which had extensive distribution ranges within South Africa. The recordings of soil type were required since an individual may have occurred in a soil pocket which differed in texture to the general soil texture of the area. Therefore the soil type could not be determined using the coordinates recorded for the place of collection and the general soil maps of South Africa.

For each specimen three factors were recorded.

- i) **Soil type.** Three categories were used, relating to the soil texture in which the specimen was growing.

Sand.

Loam.

Clay.

- ii) **Rainfall.** Three categories were used, relating to the average rainfall of the region in which the specimen was collected.

Arid: less than 400mm/annum.

Semi-arid: 400mm - 900mm/annum.

Temperate: more than 900mm/annum.

- iii) **Extent of sheath development** Six categories were used, and depended mainly on the thickness and consolidation of the sheaths. The thickness of the sheaths is not related to the age of the root nor the position along the root since, when present, sheaths (in contrast to the recordings of Leistner 1967 and McCully and Canny 1986) develop uniformly along the length of the root.

5 - Thick (greater than 3.5mm diameter), well consolidated sheath (Figure 3.1 a). Prolific root hair development which is not easily visible with the naked eye due to the excessive quantities of sand in the sheath. No secondary branching of the roots.

4 - Thick sheaths (2.5 - 3.5mm diameter), often less consolidated. Root hairs and secondary branching as for 5.

3 - Thinner sheaths (1 - 2.5mm diameter), even less consolidated (Figure 3.1 b), with more obvious root hairs. Some secondary branching of the roots.

2 - Very thin sheaths (0.75 - 1mm diameter) which are easily removed. Prolific root hairs visible with the naked eye. Secondary branching more extensive than in category 3.

1 - The beginning of sheath development, with some sand particles held by the prolific root hairs. Extensive secondary branching of the roots.

0 - None of the above sheath characteristics.

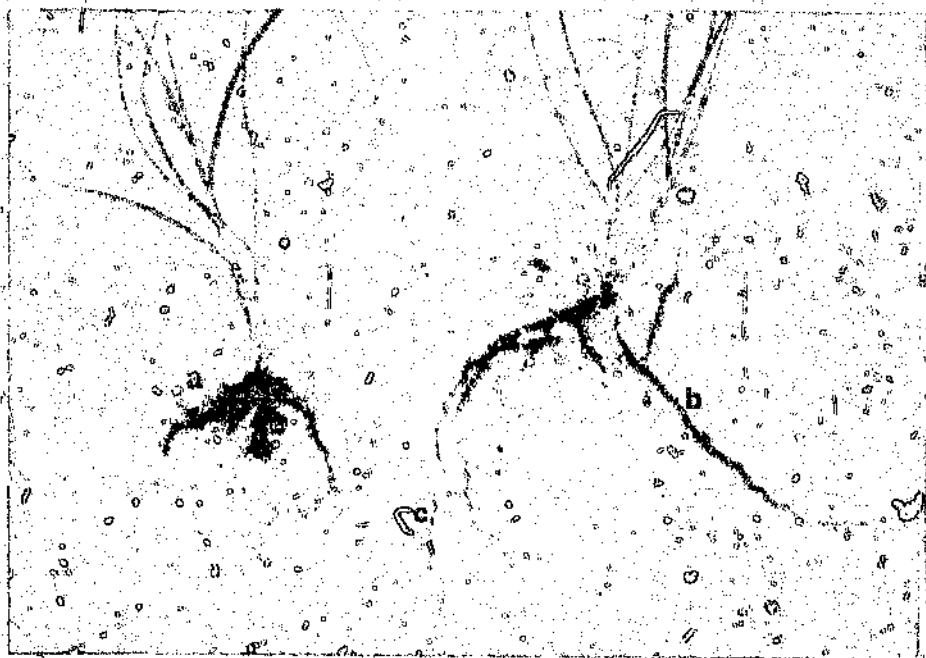


Figure 3.1. (a) Thick, well consolidated rhizosheaths of *Eragrostis pallens*. (b) Thinner, much less well consolidated sheaths, of *E. pallens*. (c) Sheath removed with root stele remaining.

Since the number of specimens per species varied, a number of classes had to be used to distinguish what proportion of the individuals in each species were in each rainfall, soil type or extent category. Six classes were used, and were determined by the percentage of the total number of specimens within the category.

Class	% of the individuals
1	1-9
2	10-29
3	30-49
4	50-69
5	70-99
6	100

In order to determine any pattern in extent of sheath development within the tribes a similarity table was constructed using the data on the percentage of individuals within each sheath extent class (Table 3.1.). The table was constructed such that the general trend down the table, from group 1 through to group 6, as well as within each group, is towards more individuals of each species with thinner sheaths. Within each group the species were arranged according to their tribes (species from the same tribe were placed together).

The aim of this study was not to determine cause and effect, but rather a relationship between sheath occurrence and one or more environmental condition(s). Therefore, by using the environmental and sheath data, a figure (Figure 3.2) was constructed to depict the relationship between the extent of sheath development and the soil and rainfall conditions.

3.4 RESULTS

3.4.1 Trends in the presence of rhizosheaths and the extent of their development within South African grasses.

Only tribes about which conclusions may be drawn concerning rhizosheath development or presence will be discussed. For example, where only one species of the tribe was studied (due to the lack of collected specimens), no conclusions about this tribe may be reached nor any trend in sheath presence detected and this tribe will not be included in this report (section 3.4.1). The description of the trend in each tribe can be further understood by referring to Table 3.1 in each case. The symbols in parentheses in the text correspond to the symbols used in Table 3.1.

Subfamily: Arundinoideae

Tribe: Aristideae (G)

All species of the two genera within this tribe had sheath development. More than 50% of the individuals of each species had medium or well developed sheaths (with a rank of at least 3). Within both genera there is a species which is divided into two varieties, both of which had similar variation in extent of sheath development amongst their individuals.

Subfamily: Bambusoideae

Tribe: Ehrharteae (?)

Of the four species of *Ehrharta* studied three did not exhibit sheath development. There was large variation in extent of sheath development between individuals of *E. calycina*.

Subfamily: Chloridoideae

Tribe: Chlorideae (*)

Members of the tribe Chlorideae formed two distinct groups. In the first group all individuals of the species had rhizosheaths and at least half the individuals had well developed sheaths (with a rank of 4 or 5). None of these species had thinly sheathed individuals. More than 70% of all individuals of the species in the second group (refer to Table 3.1 groups 3-6) were thinly sheathed (with a rank of 1 or 2), while two species did not have any sheath developing individuals.

There was a limited pattern of extent of sheath development within the genera of this tribe. All three of the *Triraphis* species occurred within the latter group, with two species exhibiting no sheath development. Five of the six *Eragrostis* species occurred within the first group.

Tribe: Pappophoreae (&)

All individuals of both genera within this tribe had sheaths. Both *Schmidtia* species had well developed sheaths (rank 4 or 5) in all individuals, although the species have different distribution ranges in terms of soil type and rainfall. All individuals of the *Enneapogon* species had either thin or medium thickness sheaths. The four species of this genus were represented on all three soil types. However, three of the species had more than half their individuals occurring in areas with an annual rainfall of 400mm-800mm, while *E. scurber* only occurred in areas with an annual rainfall of <400mm.

Subfamily: Panicoideae

Tribe: Andropogoneae (!)

This tribe had representatives within the full range of extent of sheath development, except extent 5 (refer to Table 3.1 - in all groups except group one). The sub-tribe Andropogonineae was represented by species within this large range. Genera of the sub-tribe Rottboellineae had either no sheaths (*Elionurus* and

Urelettrum) or what appeared to be the start of sheath development (*Hemarthia*). Sheaths were only well developed in *Cymbopogon marginatus* (rank 4), although more than 50% of the individuals had medium thickness sheaths. All the individuals of the other species had either thin or medium thickness sheaths. All, except three of the species (including *C. marginatus* which occurs in arid and semi-arid areas), occur in semi-arid to high rainfall areas (400mm/annum to >900mm/annum). This indicates that in drier areas the extent of sheath development is greater.

Tribe: Arundinelleae (-)

All the species exhibited very thin sheaths, except two *Polypogon* species, which had no sheath development. It was interesting to note that these two species were mostly represented in semi-arid to arid areas.

Tribe: Paniceae (#)

There was great variation in extent of sheath development within this tribe, ranging from all individuals of a species with well developed sheaths (including mainly the *Brachiaria* species), through species which had a range of sheath thicknesses on different individuals, to species which did not have any individuals which exhibited sheaths. At the genus level, the only other trend which could be found was in *Setaria*. More than half the individuals of each *Setaria* species had thin sheaths (rank 2) and the rest of the individuals exhibited medium thickness sheaths (refer to Table 3.1 - group 3).

Subfamily: Pooideae

Tribe : Aveneae (\$)

None of the species studied in this tribe had well developed sheaths and 70% or more of the individuals of each species which exhibited sheath development, had only thin sheaths (extent class 2).

Tribe: Poaceae ({})

None of these species studied had extensive sheath development (extent class 5). However, the range of extent of development was great - from no sheath development, in five species, to fairly well developed sheaths in *Festuca scabra* (extent class 4). Both *Poa* species had no sheath development. Therefore, rhizosheaths appear to be a characteristic at the species level in this tribe.

All the genera studied in the other tribes in this subfamily, Bromaceae (^), Meliceae ([) and Triticeae (~), exhibited sheath development. *Brachypodium bolusii* of the tribe Brachypodieae (*), was an exception.

From the above description it is clear that rhizosheath development is not a characteristic at any one particular taxonomic level, but varies between, as well as within, all tribes and genera.

Table 3.1. Similarity table based on extent of rhizosheath development.

EXTENT OF SHEATH DEVELOPMENT			5 4 3 2 1 0			EXTENT OF SHEATH DEVELOPMENT			5 4 3 2 1 0			EXTENT OF SHEATH DEVELOPMENT			5 4 3		
Group 1						Group 1						Group 5					
* P	<i>Eragrostis pallens</i>	6				* P	<i>Helictotrichon tuigidulum</i>	2	3	2		* P	<i>Helictotrichon longifolium</i>			2	
* A	<i>E. ciliatensis</i>	5	2			(A	<i>Lolium rigidum</i>	2	4	2		A/P	<i>Ehrharta calycina</i>			2	
* A	<i>Brachiaria byzanthia</i>	5	3			(P	<i>Cymbopogon plurinodis</i>	3	4			* P	<i>Panicum schinzii</i>			2	
* P	<i>Stipagrostis namaguensis</i>	4	4			(P	<i>Hyparrhenia dregeana</i>	3	4			* P	<i>Chloris virgata</i>			2	
* P	<i>S. ciliata</i> var. <i>capensis</i>	4	3			(P	<i>Urochloa mosambicensis</i>	3	4			* P	<i>Cynodon incompletus</i>			2	
A/P	<i>Pogonarthria squarrosa</i>	4	3			* P	<i>Panicum coloratum</i>	3	4			* P	<i>C. dactylon</i>			2	
* P	<i>Eragrostis obtusa</i>	4	3			* P	<i>Setaria nigrirostris</i>	3	4			* P	<i>Sporobolus virginicus</i>			2	
* P	<i>E. curvula</i>	3	5			* P	<i>S. sphacelata</i> var. <i>sphacelata</i>	3	4			* A	<i>S. pyramidalis</i>		2	2	
* P	<i>Sporobolus tioclados</i>	3	4			* P	<i>S. vorticillata</i>	3	4			* P	<i>Digitaria monodactyla</i>				
* P	<i>Schmidtia pappophoroides</i>	3	4			* P	<i>Phalaris minor</i>	3	4			* P	<i>Danthoniopsis parva</i>				
* P	<i>Brachiaria serrata</i>	3	4			* P	<i>Setaria sphacelata</i> var. <i>torita</i>	2	5			* P	<i>Loudetia simplex</i>				
* P	<i>B. nigropodata</i>	2	5			* P	<i>S. incrassata</i>	2	5								
* P	<i>Antheropogon pubescens</i>	2	5			* P	<i>Digitaria tornata</i>	2	5								
* P	<i>Sporobolus imbricatus</i>	2	5			(P	<i>Bothriochloa insculpta</i>	2	5								
* P	<i>S. nitens</i>	2	5			(P	<i>Cymbopogon validus</i>	2	5								
* P	<i>Panicum douglasii</i>	4	2	2		(P	<i>Hyparrhenia suprema</i>	1	5								
A/P	<i>P. maximum</i>	2	3	3		(P	<i>S. filipendula</i> var. <i>pilosa</i>	1	5								
* P	<i>Fingerhuthia africana</i>	6	6			(P	<i>Enneapogon scoparius</i>	1	5								
* P	<i>Schmidtia katheriensis</i>	6	6			(P	<i>Urochloa brachyura</i>	1	5								
* P	<i>Stipagrostis uniplumis</i> var. <i>neesii</i>	6	6			(A/P	<i>Agrostis lachnantha</i> var. <i>lachnantha</i>	1	5								
* A	<i>S. uniplumis</i> var. <i>uniplumis</i>	6	6			(A	<i>Vulpia fasciculata</i>	6	6								
* P	<i>S. obtusa</i>	5	2			(A	<i>Triraphis purpurea</i>	6	6								
* P	<i>Aristida diffusa</i> subsp. <i>butleri</i>	5	2			(P	<i>Bothriochloa hirsuta</i>	6	6								
* P	<i>A. diffusa</i> subsp. <i>diffusa</i>	4	4			(P	<i>Schizachyrium</i>	6	6								
* P	<i>Cynodon hirsutus</i>	4	4			(P	<i>S. arvensis</i>	6	6								
* P	<i>Flagrostis capensis</i>	4	3			(P	<i>Hyparrhenia hirta</i>	6	6								
Group 2						Group 4						Group 6					
* A	<i>Bromus diandrus</i>	3	4			(P	<i>Eragrostis racemosa</i>	3	4								
* P	<i>Aristida vestita</i>	3	4			(P	<i>Urochloa panicoides</i>	3	4								
* P	<i>Cymbopogon marginatus</i>	3	4			(P	<i>Hemarthra altissima</i>	3	4								
* P	<i>Enneapogon cenchroides</i>	2	4			(P	<i>Melica decumbens</i>	3	4								
(P	<i>Festuca scabra</i>	2	4			(P	<i>Bromus leptocladus</i>	3	4								
* P	<i>Digitaria eriantha</i>	1	4			(P	<i>Polypogon monspeliensis</i>	3	4								
* P	<i>D. natalensis</i>	1	4			(P	<i>Agrostis eriantha</i> var. <i>eriantha</i>	2	5								
* P	<i>Urochloa oligotricha</i>	5	2			(P	<i>Tristachya leucothrix</i>	2	5								
* A	<i>Bromus pectinatus</i>	6	6			(A	<i>Vulpia myuros</i>	2	5								
* P	<i>Enneapogon pteroriensis</i>	6	6			(A	<i>V. muralis</i>	2	5								
* P	<i>E. scaber</i>	6	6			A/P	<i>Lolium multiflorum</i>	6	6								
(P	<i>Cymbopogon prolimis</i>	6	6			(P	<i>Loudetia flava</i>	6	6								
(P	<i>Stipa dregeana</i> var. <i>dregeana</i>	6	6			(P	<i>Tristachya rhemannii</i>	6	6								
(P	<i>Hordeum murinum</i> subsp. <i>glaucom</i>	6	6			(P	<i>Hordeum capense</i>	6	6								
(P	<i>H. murinum</i> subsp. <i>leporinum</i>	4	4			(P	<i>Stachypodium distachyon</i>	6	6								
(P	<i>H. murinum</i> subsp. <i>murinum</i>	4	3			(P	<i>H. flexum</i>	6	6								
(P	<i>Aristida congesta</i> subsp. <i>congesta</i>	4	3			(P	<i>Phalaris arundinacea</i>	6	6								
(P	<i>A. junceaformis</i> subsp. <i>junceaformis</i>	4	3			(P	<i>Helictotrichon hirtulum</i>	6	6								
(P	<i>Bothriochloa radicans</i>	4	3														
Group 2						Group 4						Group 6					
* A	<i>Bromus diandrus</i>	3	4			(P	<i>Eragrostis racemosa</i>	3	4								
* P	<i>Aristida vestita</i>	3	4			(P	<i>Urochloa panicoides</i>	3	4								
* P	<i>Cymbopogon marginatus</i>	3	4			(P	<i>Hemarthra altissima</i>	3	4								
* P	<i>Enneapogon cenchroides</i>	2	4			(P	<i>Melica decumbens</i>	3	4								
(P	<i>Festuca scabra</i>	2	4			(P	<i>Bromus leptocladus</i>	3	4								
* P	<i>Digitaria eriantha</i>	1	4			(P	<i>Polypogon monspeliensis</i>	3	4								
* P	<i>D. natalensis</i>	1	4			(P	<i>Agrostis eriantha</i> var. <i>eriantha</i>	2	5								
* P	<i>Urochloa oligotricha</i>	5	2			(P	<i>Tristachya leucothrix</i>	2	5								
* A	<i>Bromus pectinatus</i>	6	6			(A	<i>Vulpia myuros</i>	2	5								
* P	<i>Enneapogon pteroriensis</i>	6	6			(A	<i>V. muralis</i>	2	5								
* P	<i>E. scaber</i>	6	6			A/P	<i>Lolium multiflorum</i>	6	6								
(P	<i>Cymbopogon prolimis</i>	6	6			(P	<i>Loudetia flava</i>	6	6								
(P	<i>Stipa dregeana</i> var. <i>dregeana</i>	6	6			(P	<i>Tristachya rhemannii</i>	6	6								
(P	<i>Hordeum murinum</i> subsp. <i>glaucom</i>	6	6			(P	<i>Hordeum capense</i>	6	6								
(P	<i>H. murinum</i> subsp. <i>leporinum</i>	4	4			(P	<i>Stachypodium distachyon</i>	6	6								
(P	<i>H. murinum</i> subsp. <i>murinum</i>	4	3			(P	<i>H. flexum</i>	6	6								
(P	<i>Aristida congesta</i> subsp. <i>congesta</i>	4	3			(P	<i>Phalaris arundinacea</i>	6	6								
(P	<i>A. junceaformis</i> subsp. <i>junceaformis</i>	4	3			(P	<i>Helictotrichon hirtulum</i>	6	6								
(P	<i>Bothriochloa radicans</i>	4	3														

Within the range of grass species studied six groups may be distinguished in terms of the presence of rhizosheaths and the extent of their development (Table 3.1).

- i) The first group consists of species which had at least half their individuals exhibiting well developed rhizosheaths (rank 4 or 5).

It is essential to note here that the species within this group have wide distribution ranges with respect to soil and rainfall conditions.

- ii) The species in the second group had at least half, if not all, their individuals exhibiting a medium degree of sheath development (rank 3).

- iii) In the third group at least half, if not all, the individuals of the species had thin rhizosheaths (rank 2).

- iv) In the fourth group at least half the individuals of each species had what appeared to be the start of rhizosheath development (rank 1). In this and all of the above groups, none of the species had non-sheath forming individuals.

The individuals in this group (iv) usually grew in sandy soil.

- v) The species in the fifth group had a varied distribution of extent of sheath development amongst their individuals, including a high percentage of individuals of some species with no sheath development.

- vi) The sixth group consists of species in which sheath development was completely absent.

It is important to note here, that most of the species in this group (vi) did not have representative individuals in

arid areas. Those that do, have only less than 30% of their individuals in these areas.

It is evident that sheath development is not restricted to perennial species (P in Table 3.1), since over a quarter of the sheath forming species studied were annuals (A in Table 3.1). Eighty two percent of the perennial species were sheath forming and the same percent of the annual species were sheath forming.

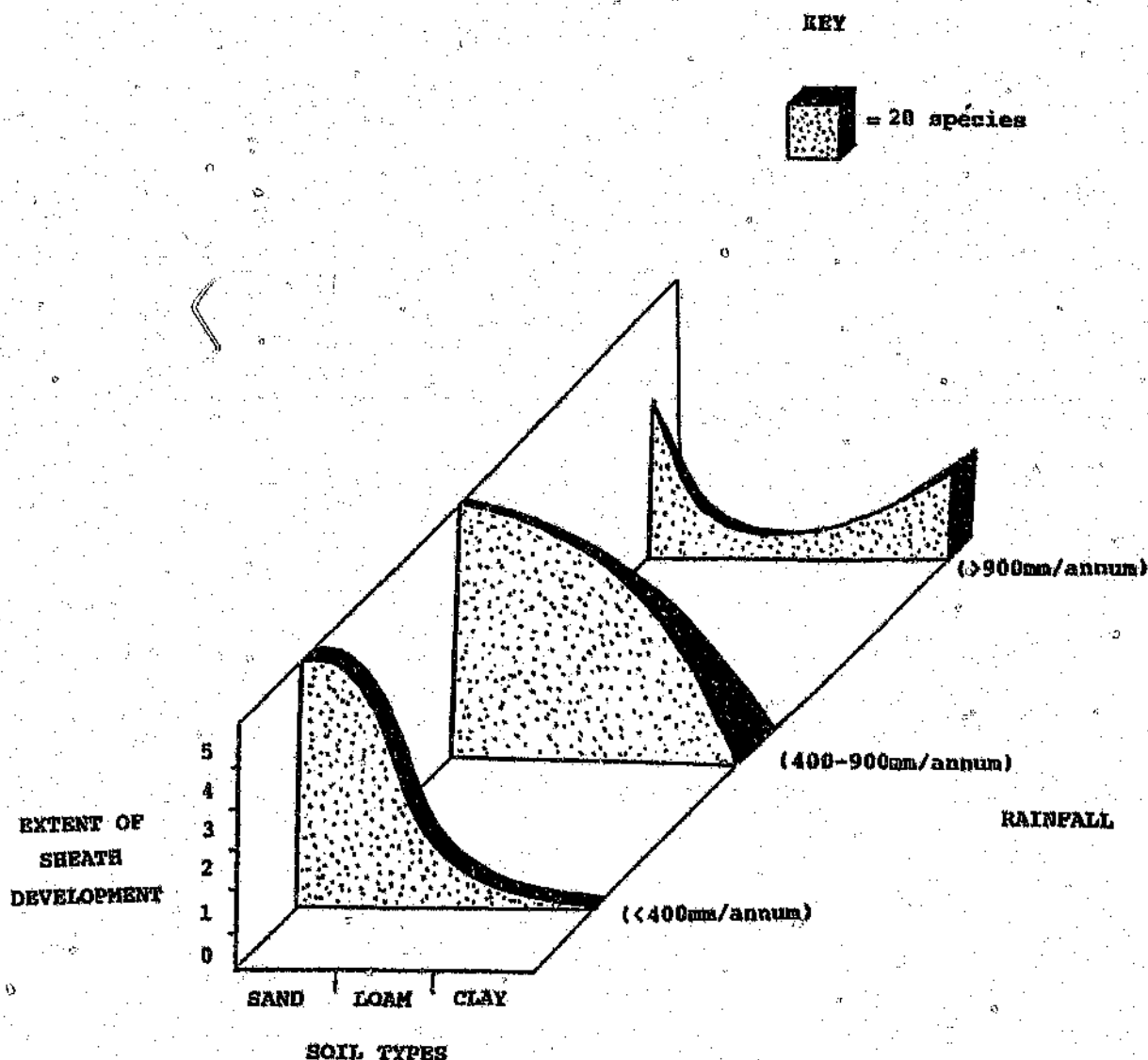


Figure 3.2. General distribution of species in terms of soil and rainfall conditions and extent of sheath development.

Figure 3.2 shows that the development of rhizosheaths is not restricted to sandy soil nor to arid areas.

There is a high representation of species in sandy, arid (rainfall less than 400mm/annum) areas and in these species the extent of sheath development is generally large. There is also a high representation of species in semi-arid areas (rainfall 400-900mm/annum), especially on clay soil. In both these rainfall areas the individuals in the sandy soil regions generally have more extensive sheath development than those in clay soil. These trends indicate a relationship between the extent of sheath development and soil texture.

The average extent of sheath development in sandy soil in high rainfall (rainfall exceeds 900mm/annum) areas is lower than that in arid and semi-arid areas. Within high rainfall areas, there is a similar extent of sheath development between individuals on the different soil types and a high number of species without sheaths. These trends show that a relationship between extent of sheath development and rainfall conditions exists, especially within individuals in sandy soil.

3.5 DISCUSSION

3.5.1 Presence of rhizosheaths in South African grasses.

Rhizosheath development occurs in most South African grass species. Only 18% of the species studied did not exhibit rhizosheaths, but these species are distributed within more than half the tribes. In contrast to Leistner's (1967) recordings, but in agreement with Duell and Peacock's (1985) recordings, rhizosheath development is not restricted to perennial species and only 18% of the annual species considered in this study did not exhibit sheath development. Also in contradiction to previous recordings (McCully and Canny 1986), the sheaths extend over the entire length of all the roots of any sheath forming

grass plant.

Assuming that sheaths influence root functioning and microbial activity (refer to chapter 2, section 2.9) the below-ground productivity of grasslands would be influenced by rhizosheaths since, the sheaths are common in grasses and the distributions of sheath forming species are wide, encompassing all rainfall and soil conditions.

Sheath presence does not distinguish any specific tribe(s) since each tribe has representative sheath forming species. The data do suggest that sheaths may be a characteristic of the tribes Aristideae, Bromaeae, Meliceae, Pappophoreae and Triticeae. In other words sheaths are present on all individuals of all species within these tribes. However, in order to state this as a definite conclusion more specimens would have to be collected for each species.

Except for *Arundinella*, *Elionurus*, *Trichopteryx* and *Urelytrum*, each of which has only one species, and *Leersia*, of which only one species was studied, all the genera considered in this study had representative sheath forming species. Therefore, as is the case for tribes, sheath presence is not a characteristic which can be used to distinguish between different genera. In addition, sheath presence does not distinguish between different varieties, because when two varieties of a species were studied no difference in the presence or extent of sheath development occurred.

Although it has been shown that sheaths are present in most grass species, the extent of development varies greatly between species and sometimes even within species. The most extensive development of rhizosheaths occurs in species within only four tribes - Aristideae, Chlorideae, Paniceae and Pappophoreae, two of which, Chlorideae and Paniceae, have considerable variation in extent of development between their genera.

Unfortunately, due to the lack of a sufficient number of herbarium specimens with adequate information on soil conditions in which the specimen grew, only limited conclusions may be drawn with respect to whether sheaths are a distinguishing feature of any genera or species. It was initially thought that the sheath forming characteristic may be included as an important, easily observed trait in Poaceae species field identification keys. However, this would have been possible only if the development of the sheaths was genetically fixed and did not vary depending on the environmental conditions. The constancy with which sheaths are found within a genus or a species varies between genera and species. In certain genera the different species develop sheaths to different extents but the extent of development is uniform between all individuals within the respective species. Within such genera, for example *Cymbopogon*, *Festuca*, *Panicum* and *Schizachyrium*, sheath presence may be a distinguishing feature between species, within a genus, which may be confused on initial examination of field specimens or when only vegetative material is available for identification. The success of this method of species identification will vary between genera. Distinction between species which develop extensive sheaths under all environmental conditions (extent 4, 5 and possibly 3), and those without sheaths will be the most useful. This distinction may be most successful between *Cymbopogon excavatus* (no sheath development) and *C. marginatus* (extent 3 or 4), where the vegetative and reproductive structures are similar. Sheath presence on *C. prolixus* (extent 3) may make the distinction between this species and *C. excavatus* easier, especially when only vegetative material is available for identification. Likewise, *Panicum maximum* and *P. natalense* (no sheath development) may be distinguished by the extensive sheath development on *P. maximum* (extent 3, 4 or 5), when only vegetative material is available for identification.

3.5.2 Sheath presence as a genetically fixed trait as opposed to the extent of development as a facultative response to the environment.

It must be emphasised that there is a difference between the presence of sheaths and the extent to which they develop. No relationship between the presence of rhizosheaths and environmental conditions was found. If sheaths occurred on one individual of a species then all individuals of that species exhibited sheath development irrespective of where the individuals grew (with the exception of six species, and this will be discussed later). It can be concluded that the presence of rhizosheaths is a genetically fixed trait. However, as opposed to what the pilot survey indicated, sheath presence on one species of a particular genus does not guarantee sheath presence on all the species of that genus.

Although present in the majority of South African grass species, the sheath developing trait is apparently absent in certain species. The species which do not have this trait occur within group 6 of Table 3.1, i.e. those which never develop rhizosheaths regardless of the environmental conditions, and include members of more than half of the tribes studied.

Within the range of species in which the sheath forming trait is present, two conditions exist in terms of the extent to which the sheaths develop.

- i) The extent to which sheaths develop is fairly uniform between individuals within each separate species, irrespective of the environmental conditions in which they grow (groups 1-4 in Table 3.1). This is not to say that the extent to which the sheaths develop does not differ between species. Most of these species had representative individuals in all the rainfall and soil type categories. This is an indication that the extent to which sheaths develop in these species is possibly also a genetically

fixed trait.

- ii) The extent to which sheaths develop varies greatly between individuals of the same species (group 5 in Table 3.1). Therefore, while the sheath forming trait is present in all individuals, the extent to which it is manifest is probably a facultative response to the environmental conditions in which the individual grows. The degree of plasticity of this response varies between species, to the extent that individuals of some species (namely those in the Chlorideae and Paniceae tribes), may not exhibit any rhizosheath characteristics when growing under certain environmental conditions.

The detailed genetic control over sheath development has not been investigated. Within the species which always have the same extent of sheath development, the sheath forming trait may influence the number of epidermal hairs produced and thereby control sheath development. Another possibility is that the number of epidermal hairs produced is controlled separately and thus the genetic control over sheath development is indirect.

3.5.3 The relationship between environmental conditions and sheath presence/extent of sheath development.

Sheaths occur on individuals which grow in semi-arid and temperate conditions as well as those that grow in clay and loam soil. Therefore the hypothesis, that the development of a rhizosheath is a facultative response to arid and/or sandy conditions, must be rejected. However, the extent to which the sheaths develop is apparently often a facultative response to environmental conditions. Species found in dry, sandy areas usually have extensive sheath development relative to the average sheath development of species found in more temperate areas and clayey soils. This may account for why rhizosheaths have mainly been recorded in xeromorphic species growing in sandy soil. The less consolidated sheaths on any specimens studied in the higher

rainfall/clay soils may not have been observed or may have been removed by the collector before pressing.

Species with consistently well developed sheaths have wide distribution ranges with respect to soil and rainfall conditions. This may be a result of their increased tolerance (due to sheath presence) of low water availability. This idea is further substantiated by the fact that most of the species which do not develop rhizosheaths are absent in arid areas.

Many individuals in which sheath development was only just detected, occurred in sandy soil. This suggests some relationship between the initiation of the expression of the sheath trait and soil texture.

Although extensive sheath development is not exclusively found on individuals in sandy soil the relationship between extent of development and soil texture is evident from Figure 3.2. It is proposed that there may be a response, in terms of an increased extent of sheath development, to increased sandy soil conditions. In order to substantiate this hypothesis, controlled growth experiments were necessary.

The average extent of sheath development on sandy soil individuals in high rainfall areas is smaller than in lower rainfall areas. A similar relationship between rainfall and sheath thickness was observed by Leistner (1967) in the sandy Kalahari areas. This relationship between extent of development and rainfall suggests that, (assuming sheaths function to compensate for low water and nutrient availability as will be discussed in chapter 5), in high rainfall areas the frequently replenished soil water decreases the need for better developed sheaths. This would be particularly pertinent to sandy soil, where the water retention and nutrient availability is low relative to clay soil. In order to test this theory controlled growth experiments were conducted.

CHAPTER 4

ENVIRONMENTAL INFLUENCE ON THE EXTENT OF RHIZOSHEATH DEVELOPMENT.

4.1 INTRODUCTION

It has thus far been established that the presence of sheaths is a genetically fixed trait, present in most South African grass species. On the other hand, the extent to which the sheaths develop is often a facultative response to the environment. Therefore, the following studies will deal with the extent of development, rather than the presence, of sheaths.

Under natural conditions plants are subjected to different edaphic and climatic factors. The most obvious factors which affect root growth are those in the soil, and primarily include texture, water and nutrient availability. Different soil types (textures) have inherently different water and nutrient availability. Since it thus far appears that there is a relationship between extent of sheath development and environmental conditions, particularly soil texture and water availability, it was decided to conduct controlled growth experiments to determine whether this relationship was based on a facultative response directly to soil texture or to water and/or nutrient availability.

With respect to this, two important questions were addressed in this section of the study.

- i) Is the extent to which sheaths develop a facultative response directly to soil texture?
- ii) Is the extent to which sheaths develop a facultative response to soil water and/or nutrient availability?

It is difficult to separate the effects of soil texture and the corresponding water and nutrient availability. The more sandy the soil the shorter the period of water and nutrient availability. The greater the clay content the stronger the attractive forces between soil particles and the water, which increases the difficulty with which the water is extracted. The higher the sand content in the soil the lower the cation exchange capacity (CEC) and the higher the degree of nutrient leaching from the soil, but the easier the roots are able to extract any remaining nutrient ions from the soil.

In order to separate the effects of soil texture and the effects of soil water/nutrient status two growth experiments were carried out. The first experiment was concerned with the response to soil texture, and therefore the water availability was controlled while the texture was varied. The nutrient availability could not be controlled due to the different CEC in the different soil texture treatments. The second growth experiment was concerned with water and nutrient availability, and therefore these factors were varied while the soil texture was kept constant.

4.2 THE EXTENT OF RHIZOSHEATH DEVELOPMENT AS A RESPONSE TO SOIL TEXTURE.

4.2.1 HYPOTHESIS

It was hypothesised that the development of rhizosheaths would be more extensive the higher the sand content in the soil.

4.2.2 MATERIALS AND METHODS

Three indigenous grass species were used in this study, *Eragrostis pallens* Hack, *Antheophora pubescens* Nees, and *Digitaria eriantha* Steud. The first two species were found, during the herbarium study, to consistently develop distinct rhizosheaths, while the latter was found to have greater variability in the extent of sheath development depending on the soil and rainfall conditions under which the individuals grew.

4.2.2.1 Experimental design

Seeds of these species were grown in 11 pots in a controlled-climate growth chamber using a 14 hour day (65-80 $\mu\text{mol}/\text{m}^2/\text{s}$ quantum flux rate) at approximately 25°C followed by 10 hours of darkness at approximately 16°C. The pots were randomised weekly to eliminate differences due to position in the growth chamber.

Five sets of plants were used, each set was grown in a different soil texture treatment.

Treatment	% sand	% clay
1	80	20
2	70	30
3	60	40
4	50	50
5	30	70

Each treatment consisted of five pots/replicates, of between ten and sixteen individuals per replicate. The sand (with a 80% sand 20% clay content) was collected from a broadleaf savanna area in which *E. pallens* and *D. eriantha* grow. Kaolin powder was added to the sand to make up the clay fraction. The texture was accurately determined using a Bouyoucos hydrometer (Day 1965).

The field capacity for each soil treatment was calculated prior to the experiment and the plants were watered regularly such that

the soil was kept at 70% field capacity. It was necessary to water the sandy soil pots more frequently than the clayey soil pots because the latter drained faster. The plants were not supplied with nutrients in the water. The nutrient concentration in the different soil treatments could not be controlled, since the amounts of nutrients remaining in the soil after the water had drained through could not easily be monitored. The difference in nutrient availability between the soil textures due to the different CEC could not be controlled.

After twelve weeks growth the plants were uprooted and the presence and extent of rhizosheath development was scored for each individual using the same scale as in the herbarium study (section 3.3). Differences in extent of sheath development were not physically quantified by weighing the sheath soil since it was not possible to extract all the roots of the individuals in the clayey soil. Therefore the data could not be statistically analyzed.

4.2.3 RESULTS AND DISCUSSION

The responses to the different soil treatments varied between species, but the general trends were similar (Table 4.1 and Table 4.2). The variability in the extent of sheath development within the species was very low. The texture of the soil influenced the extent of rhizosheath development. This is in contrast to Duell and Peacock's (1985) recordings, where the soil texture did not appear to affect rhizosheath development.

In each species the greatest extent of sheath development occurred in the treatment with the highest sand content (80% sand). In *A. pubescens* and *D. eriantha* the extent of sheath development decreased with decreasing sand content until 60% sand, with further decrease in sand content the extent of development did not decrease. The extent of sheath development in *E. pallens* continued to decrease with the decrease in sand

content below 60%. In *A. pubescens* and *D. eriantha*, similar numbers of epidermal hairs were produced in the soil treatments containing 30-60% sand (Table 4.1). In *E. pallens* individuals the number of epidermal hairs decreased from the 40% sand treatment to the 30% sand treatment. As explained previously (chapter 2), the similar numbers of epidermal hairs would result in similar numbers of particles being incorporated into the sheath, and being held equally strongly. This would explain why the sheaths of *A. pubescens* and *D. eriantha* do not further decrease in extent in soil containing less than 60% sand, while *E. pallens* individuals in the last treatment had thinner sheaths.

Table 4.1. The extent of rhizosheath development, average number of epidermal hairs and average number of secondary root branches, which occurred in A) *A. pubescens*, B) *D. eriantha*, and C) *E. pallens* under the different soil texture treatments.

A)

<i>A. pubescens</i>	Description
Treatment 1 (80% sand)	
Extent	70% with extent 5 - root and sheath at least 5.5mm thick. Well consolidated sheaths. 30% with extent 4 - sheaths less consolidated.
Epidermal hairs	Prolific. Average length: 0.2mm Average number per centimetre of root: 75.
Secondary branches	Average number per centimetre of root: 8
Treatment 2 (70% sand)	
Extent	4 - root and sheath 4 - 5.5mm.
Epidermal hairs	Prolific. Average number per centimetre of root: 67. Average length: 0.2mm.
Secondary branches	Average number per centimetre of root: 11
Treatment 3 (60% sand)	
Extent	2 - average root and sheath thickness: 2.75mm
Epidermal hairs	Average number per centimetre of root: 55
Secondary branches	Average number per centimetre of root: 10
Treatment 4 (50% sand)	
Extent	As for treatment 3, except less sand and silica particles were held
Epidermal hairs	As for treatment 3.
Secondary branches	As for treatment 3.
Treatment 5 (30% sand)	
Extent	As for treatment 4
Epidermal hairs	As for treatment 3.
Secondary branches	Average number per centimetre of root: 13

Table 4.1 cont _ _ _

B)

<i>D. eriantha</i>	Description
Treatment 1 (80% sand)	
Extent	20% with extent 4 - average root and sheath thickness: 4.5mm thick. 80% with extent 3 - average root and sheath thickness: 3mm thick. Sheaths not well consolidated.
Epidermal hairs	Less prolific than in other two species. Average number per centimetre of root: 11. Average length: 0.2mm.
Secondary branches	More prolific than in the other two species. Average number per centimetre of root: 20.
Treatment 2 (70% sand)	
Extent	2 - root and sheath 2.75 - 2.9mm thick
Epidermal hairs	Average number per centimetre of root: 8.
Secondary branches	Average number per centimetre of root: 27
Treatment 3 (60% sand)	
Extent	1 - average root and sheath thickness: 2mm
Epidermal hairs	Sparse. Average number per centimetre of root: 5.
Secondary branches	Average number per centimetre of root: 15. Tertiary branching.
Treatment 4 (50% sand)	
Extent	1 - not many sand or silica particles held.
Epidermal hairs	As for treatment 3.
Secondary branches	As for treatment 3.
Treatment 5 (30% sand)	
Extent	As for treatment 4.
Epidermal hairs	As for treatment 4.
Secondary branches	More prolific. Average number per centimetre of root: 23. Tertiary branching.

Table 4.1 cont. — — —

c)

<i>E. pallens</i>	Description
Treatment 1 (80% sand)	
Extent	5 - root and sheath at least 5.5mm thick. Sheaths well consolidated.
Epidermal hairs	Prolific. Average number per centimetre of root: 100. Average length: 0.66mm.
Secondary branches	Sparse. Average number per centimetre of root: 3.
Treatment 2 (70% sand)	
Extent	4 - average root and sheath thickness: 4mm. Less consolidated than in treatment 1.
Epidermal hairs	Prolific. As for treatment 1.
Secondary branches	None.
Treatment 3 (60% sand)	
Extent	3 - average root and sheath thickness: 3mm. Less consolidated than in treatment 2.
Epidermal hairs	Average number per centimetre of root: 60
Secondary branches	None.
Treatment 4 (50% sand)	
Extent	As for treatment 3.
Epidermal hairs	As for treatment 3.
Secondary branches	None.
Treatment 5 (30% sand)	
Extent	2 - average root and sheath thickness: 2.5mm. Sheaths not well consolidated.
Epidermal hairs	Average number per centimetre of root: 45.
Secondary branches	Average number per centimetre of root: 10.

Table 4.2. Summary of the differences in extent of sheath development between the five different soil texture treatments.

	Treatment		Extent of sheath development		
	% sand	% clay	<i>A. pubescens</i>	<i>D. eriantha</i>	<i>E. pallens</i>
1)	80	20	5	3	5
2)	70	30	4	2	4
3)	60	40	2	1	3
4)	50	50	2	1	3
5)	30	70	2	1	2

The trend with regard to the change in number of epidermal hairs in response to the sand content of the soil is similar to the trend in extent of sheath development. In all three species (except *E. pallens* individuals in treatment 2), where the extent of sheath development decreased, there was a corresponding decrease in the number of epidermal hairs (Table 4.1). This finding is in accordance with previous findings which show the density of epidermal hairs to be greater in dry, sandy soil (Kutschera 1960 in Leistner 1967). It has been observed that roots in sandy soil produce more epidermal hairs than roots of the same species in less sandy soil. The sand particles may mechanically stimulate the production of root hairs by abrasion of the epidermis (M. Fey personal communication). Another possibility is that the rapid filtration of water through the soil may necessitate an increased root surface area for adequate water absorption, hence the increased root hair production. The general trend in the change in number of secondary branches was that the smaller the extent of sheath development, the more secondary branches that were produced.

The relationship between extent of sheath development and sand content in the soil is evidence to support hypothesis 4.2.1, that

the more sandy the soil the greater the extent of sheath development.

As explained in section 4.1, these results may be confounded by the different nutrient availabilities inherent in the different soil treatments. To determine whether the extent of development was a response to soil conditions other than texture another growth study was conducted wherein the soil texture effect was standardized.

4.3 THE EXTENT OF RHIZOSHEATH DEVELOPMENT AS A RESPONSE TO WATER AND NUTRIENT AVAILABILITY.

The findings of the herbarium study and the observations of Leistner (1967) indicate that the extent of sheath development may be a response to low water, rather than to soil texture.

4.3.1 HYPOTHESIS

The second hypothesis, with respect to environmental influence on the extent of rhizosheath development, was that sheath development would be more extensive on individuals growing in conditions of low water and/or low nutrient availability than on individuals growing in conditions of high water and high nutrient availability.

4.3.2 MATERIALS AND METHODS

4.3.2.1 Experimental design

Seeds of the same three species were grown in the same temperature and light conditions as in the previous experiment. The plants were grown in 11 pots containing sandy soil (80% sand, 20% clay) collected from the same area as before. The pots were randomised weekly to eliminate differences due to position. Four sets of plants were used, each set was grown under different

combinations of water and nutrient availability. Ten replicates (between ten and sixteen individuals per replicate) in each set/treatment were grown.

Treatment	Water	Nutrients
1	+	+
2	-	+
3	+	-
4	-	-

Plants in treatments 1 and 2 were watered with a Long-Ashton solution, containing all essential micronutrients, iron, 40ppm phosphorus ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) and 50ppm nitrogen (KNO_3 and $(\text{NH}_4)_2\text{SO}_4$; $1\text{NO}_3^-:2\text{NH}_4^+$). Plants in treatments 3 and 4 were watered with a similar Long-Ashton solution excluding the phosphorus and nitrogen. Plants in treatments 1 and 3 were kept at 70% field capacity while those in treatments 2 and 4 were kept at 30% field capacity in order to subject the plant to some degree of water stress. The leaves of these plants (in treatment 2 and 4) were slightly curled but the plants did not appear to be completely drought stressed.

After the plants had grown for twelve weeks, and a sufficient root mass had developed, individuals from five replicates of each treatment were uprooted and the extent of sheath development and the mass of the sheath soil was recorded for each individual. It was possible to do this in this experiment because of the sandy nature of the soil. This procedure was repeated on the other five replicates after a further four weeks growth.

4.3.2.2 Statistical analyses

The sheath soil mass data were normally distributed and therefore analysis of variance tests were conducted on this data to determine whether there was significant variation within the

data. In order to determine between which treatments the differences occurred, and whether differences occurred within a treatment over time, Tukey tests were performed on the data. Tukey tests are multiple comparisons which show which treatment means are different from which other treatment means (Miller 1981; Miller 1985).

4.3.3 RESULTS AND DISCUSSION

Table 4.3. Differences in extent of sheath development and sheath soil mass between the different water/nutrient treatments (mean values, $\bar{x}$, and standard deviations, sd, of the five replicates are given).

Treatment		Extent of sheath development		Sheath soil mass (g)		Sig. of mass change	
water	nutrients	3mths	4mths	3mths $\bar{x}$ sd	4mths $\bar{x}$ sd		
<i>Anthephora pubescens</i>							
+	+	3	4	.05 ^a	.00	.10 ^a	.00 **
-	+	2	3	.04 ^b	.01	.08 ^b	.00 **
+	-	2	1	.04 ^b	.01	.02 ^c	.00 **
-	-	1	1	.01 ^c	.00	.01 ^c	.00 NS
<i>Digitaria eriantha</i>							
+	+	3	4	.04 ^a	.01	.09 ^a	.01 **
-	+	2	3	.02 ^b	.00	.01 ^b	.00 NS
+	-	2	1	.01 ^c	.00	.01 ^b	.00 NS
-	-	1	3	.01 ^c	.00	.03 ^c	.01 **
<i>Eragrostis pallens</i>							
+	+	5	5	.56 ^a	.06	1.39 ^a	.07 **
-	+	3	5	.14 ^b	.01	.74 ^b	.08 **
+	-	4	4	.29 ^c	.01	.30 ^c	.01 NS
-	-	3	4	.11 ^b	.02	.18 ^c	.03 NS

Data in the table with different superscripts are significantly ($p < 0.05$) different from each other (this applies to only within-species comparisons and within each age group).

Individuals of each species which grew in conditions of high water and nutrient availability had the most extensive sheath development and significantly ($p < 0.05$) more soil incorporated in their sheaths than all the other individuals. The extent of sheath development in *E. pallens* was ranked the same in individuals in treatment 1 and 2, however if the designated ranks went higher the individuals in treatment 1 would have been ranked more than 5 (as indicated by the soil mass data). All the individuals in the high nutrient treatments increased the extent of sheath development during the fourth month and all these individuals, (except *D. ariantha* individuals in treatment 2), significantly ($p < 0.05$) increased the amount of soil incorporated in their sheaths over time. Within the individuals in high water, low nutrient treatments the extent of sheath development either decreased over time or remained constant and no more soil particles were incorporated into the sheath. Within the individuals in low water, low nutrient treatments the extent of sheath development either increased over time or remained constant.

Although there is a relationship between the extent of sheath development and soil nutrient and water availability, it is an inverse relationship to that expected at the outset of the experiment. The hypothesis that individuals growing in conditions of low water and/or low nutrient availability would exhibit the most extensive sheath development, must be rejected. These results also conflict with the findings of Duell and Peacock (1985), that soil fertility has no effect on rhizosheath development. However, these researchers probably only considered whether sheaths were present or absent.

4.4 OVERALL DISCUSSION.

The results of the two experiments provide evidence for the conclusion that, in the species studied, an increase in the extent of rhizosheath development is a direct response to an increase in sand content in the soil. The greatest extent of development occurred on individuals growing in soil with the highest sand content (80% sand), while variation in the extent of development occurred between individuals growing in this soil (80% sand) under different conditions of nutrient and water availability. The least extensive development occurred on individuals in the sand with low water, low nutrient availability. Therefore, the more extensive sheath development in sandy soil is not a response to the lower water and lower nutrient availability of this soil relative to clay.

These trends occurred in all three species, including *E. pallens*, which has only been collected from sandy soil areas. It is therefore proposed that similar trends would occur in all grass species which possess the sheath forming trait.

None of the individuals which were grown in conditions of high water, low nutrient availability, increased the extent of their sheath development over time. On the other hand, it is important to note that most of the individuals which were grown in conditions of low water availability generally increased the extent of sheath development and often significantly increased the amount of soil incorporated in the sheath over time. These individuals probably grow slower than individuals in high water conditions. The production of epidermal hairs and exudation of mucilage would also probably be slower and therefore the development of the rhizosheath may be slower. The sheath may eventually develop to the same extent as on individuals in conditions of high water, high nutrient availability, but over a longer time. These observations may, on the other hand, infer that an increase in extent of sheath development over time may be a response to extended dry periods in sandy soil. The

increased extent of sheath development in individuals in conditions of high water, high nutrient availability may be explained by the healthy, rapid root growth which would probably result in increased sheath thickness.

Under each treatment condition individuals of *E. pallens* had more extensive sheath development than individuals of the other two species. If the sheaths function to increase the vigour of the individuals then it would appear that individuals of *E. pallens* would be at a competitive advantage over the other species, especially under stressful environmental conditions. *E. pallens* was the only species found, during the herbarium study, to have consistently well developed sheaths. However, as highlighted in chapter 3, individuals of this species had been collected only from sandy soil as they are not common on clay soil. If individuals had been collected from other soil types and in high rainfall areas, a variation in extent of sheath development may have been observed, as occurred in the experiments which manipulated these factors. The possibility of *E. pallens* being more competitive than *A. pubescens* and *D. eriantha* under all environmental conditions, if they grew sympatrically, will be further discussed in the following chapter when sheath functions are considered.

It is interesting to note that individuals of *A. pubescens* had a greater extent of sheath development in treatment 1 of the first experiment than individuals of this species in the second experiment. Individuals in these two experiments were grown in soil of the same texture (80% sand), with similar soil water contents. The only difference between these two experimental conditions was the supply of micronutrients and iron to individuals in the second experiment. The more extensive development may therefore be a response to low availability of micronutrients and/or iron. This possibility was not investigated in this study since the trend was not observed in the other two species. It could be investigated by a multifactorial experiment in which seeds of *A. pubescens* and

other species are grown in similar conditions of soil water, nitrogen and phosphorus availability, but under different combinations of iron and micronutrient availability. The results from such an experiment would lead to an even better understanding of the facultative response of sheath development to the environment.

PART III POSSIBLE FUNCTIONS OF RHIZOSHEATHS.

The key question addressed in part III of the study was: Do rhizosheaths function to increase the plant vigour and survival, particularly in low nutrient environments? Although a number of hypotheses (section 2.9) have been put forward with respect to rhizosheath functions, none of these have been conclusively tested. The numerous hypotheses were summarised into four possible functions which were then investigated in this study.

- i) Compensation by sheaths for low water availability.
- ii) Compensation by sheaths for low nutrient availability.
- iii) Influence of the extent of sheath development on the uptake of immobile nutrient ions.
- iv) Sheath influence on the microorganisms at the soil-root interface.

Although it was found that increased sheath development did not occur in response to low water and/or nutrient availability, the possibility of the sheaths functioning to compensate for low availability could not be discarded. These functions may increase plant vigour under all environmental conditions not only in conditions of low nutrient availability.

The functioning of roots is vital to the net primary productivity (NPP) of all ecosystems. In grasslands in particular, roots may be responsible for more than 60% of the NPP (Coleman et al 1976). How the roots alter the rhizosphere and influence the microbial populations in the soil has a marked effect on the secondary productivity of a system. This is particularly important in grasslands, where as much as 90% of the secondary productivity may occur as microorganisms (Stanton 1988). Both the primary and secondary productivity of an ecosystem rich in grasses may be significantly affected by the widespread occurrence of rhizosheaths and their influence on root functioning and

microorganisms.

If all or some of these functions were found to exist, they would explain how the sheathed species are suited for survival in environments where the soil water and nutrient availability is low, such as in the many semi-arid, sandy areas of South Africa.

Chapter 5 will deal with rhizosheath compensation for low water and low nutrient (nitrogen and phosphorus) availability. Sheath influence on the rate of phosphorus uptake and on microorganisms at the soil-root interface will be dealt with in chapters 6 and 7 respectively.

CHAPTER 5

SHEATH COMPENSATION FOR LOW WATER AND LOW NUTRIENT AVAILABILITY

5.1 INTRODUCTION

In soils with a low water content and low nutrient status plants may increase the proportion of their reserves that are channelled into root production. This is often at the expense of shoot production (increasing the root mass:shoot mass ratio). The more extensive the root system the greater the volume of soil from which nutrients and water may be taken up. Another method of increasing nutrient uptake in low nutrient soils is to increase mycorrhizal associations (Nye 1977). No mycorrhizal associations occur in rhizosheaths (Wullstein and Pratt 1981; Buckley 1982 and Goodchild and Myers 1987), therefore, the presence of sheaths may eliminate the need for roots to form these associations. The sheaths may also reduce the need for plants in low nutrient soils to increase their root production. Rhizosheaths may act to increase the effective circumference of the root and its absorbing area by facilitating easier water and nutrient uptake from the soil. Ions from the bulk soil dissolve readily in the sheath mucilage. This may facilitate faster uptake of the ions by the root since nutrients are taken up from solution. The presence of the sheath would increase the volume of soil from which water and nutrients could be extracted, thereby reducing the need for increased root production.

The compensation by rhizosheaths for the low availability of water, nitrogen (N) and phosphorus (P) was investigated. Nitrogen and P were chosen as the nutrients to be investigated since both are essential macronutrients for plant growth (section 2.8). It has been hypothesised that sheaths may develop in response to nitrogen stress (Wullstein and Pratt 1981). Phosphorus is an immobile nutrient ion in the soil and is

therefore not readily available for root uptake. It is possible that sheaths compensate for N and/or P stress.

5.2 HYPOTHESIS

Through their influence on the uptake of water and nutrients, the presence of rhizosheaths would allow for equivalent water/nutrient uptake and growth rates in individuals in stressful conditions and those in non-stressful conditions. No increase in the root/shoot biomass ratio is expected in individuals growing in stressful conditions.

5.3 MATERIALS AND METHODS

The *Anthephora pubescens*, *Digitaria ariantha* and *Eragrostis pallens* plants which were grown for the previous experiment (4.3.2) were used in this study.

5.3.1 Measurements taken on live plants.

On the day prior to harvesting, leaf parameters such as stomatal conductance, temperature, and relative humidity at the leaf surface, were collected using a porometer. Five leaves per replicate were measured. From these data transpiration rates were calculated. These rates were used as an index of water uptake. Water use efficiencies (WUE) were obtained by dividing the total plant biomass by the transpiration rate which gave a measurement of the amount of carbon fixed per millimole of water used. It is recognised that biomass accumulation is an integrated value over time whereas the transpiration recordings were data obtained at only one point in time.

5.3.2 Parameters measured on the harvested plants.

When the plants were harvested to assess the extent of sheath development in the three and four month old plants for the previous experiment, four parameters were measured.

- i) Below-ground biomass. The dry weight of the roots was determined by weighing the sheathed roots, then ashing them in a furnace at 100°C and weighing the remaining soil. From this a dry mass for only the root matter was calculated per individual.
- ii) Above-ground biomass. The total weight of each plant was measured after the plants had been air dried. The ratios of root to shoot biomass were calculated and used as an indication of whether individuals in stressful conditions channelled more reserves into root production (at the expense of shoot production), than individuals in non-stressful conditions.
- iii) Total nitrogen (N) and total phosphorus (P) contents in the above-ground plant parts were determined using the methods in Anderson and Ingram (1993). Since the roots were ashed in order to obtain root biomass there was insufficient root material to analyze for N and P content.
- iv) The average weekly growth rates of the individuals in each treatment were determined using the differences in the total plant biomass of the three and four month old plants.

5.3.3 Statistical analyses.

The data were normally distributed and therefore analysis of variance tests were used to determine any significant variation in the data set for each species. If significant ($p < 0.05$) variation occurred within the overall data set, then Tukey tests

were used to determine the significant between-treatment (within-species) differences.

5.4 RESULTS AND INTERPRETATION

Similar trends were found in the three and four month old plants. Therefore, to make the interpretation and explanation of the results clearer, only the data for the four month old plants will be presented.

Within each table the data with different superscripts are significantly ($p < 0.05$) different from each other (this applies only to within-species comparisons).

Table 5.1. Differences in root mass between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd, for the five replicates of each treatment are given.

Treatment	water	nutrients	Root mass (g)	
			$\bar{x}$	sd
<i>A. pubescens</i>				
1	+	+	.06 ^a	.01
2	-	+	.09 ^b	.00
3	+	-	.04 ^c	.01
4	-	-	.03 ^d	.01
<i>D. eriantha</i>				
1	+	+	.03 ^a	.01
2	-	+	.04 ^a	.00
3	+	-	.02 ^b	.00
4	-	-	.02 ^b	.01
<i>E. pallens</i>				
1	+	+	.19 ^a	.01
2	-	+	.14 ^b	.03
3	+	-	.08 ^c	.01
4	-	-	.03 ^d	.00

The average root mass per individual growing in conditions of low nutrient availability was significantly ($p < 0.05$) lower than that of individuals in conditions of high nutrient availability. Individuals from all the species grown in low water conditions, (except those from *A. pubescens* individuals in treatment 2), had a similar or significantly ($p < 0.05$) lower average root mass than those in conditions of high water, high nutrient availability. This indicates that the individuals in the low nutrient and/or low water treatments did not increase their root mass in order to maintain sufficient nutrient and water uptake for growth in low nutrient conditions or dry conditions. These results suggest

that the presence of rhizosheaths may compensate for low nutrient and low water availability.

Table 5.2. Differences in shoot biomass and total plant biomass between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd , for the five replicates of each treatment are given.

Treatment	water	nutrients	Shoot mass (g)		Total plant mass (g)		
			$\bar{x}$	sd	$\bar{x}$	sd	
<i>A. pubescens</i>							
1	+	+	.14 ^a	.02	.21 ^a	.03	
2	-	+	.25 ^b	.01	.35 ^b	.02	
3	+	-	.06 ^c	.01	.11 ^c	.02	
4	-	-	.05 ^c	.01	.09 ^c	.01	
<i>D. eriantha</i>							
1	+	+	.08 ^a	.01	.11 ^a	.02	
2	-	+	.14 ^b	.01	.19 ^b	.03	
3	+	-	.03 ^c	.01	.06 ^c	.02	
4	-	-	.03 ^c	.02	.05 ^c	.01	
<i>E. pallens</i>							
1	+	+	.30 ^a	.03	.49 ^a	.02	
2	-	+	.26 ^a	.02	.41 ^a	.03	
3	+	-	.09 ^b	.01	.17 ^b	.02	
4	-	-	.03 ^c	.01	.07 ^c	.01	

Individuals growing in conditions of low nutrient availability had a significantly ($p < 0.05$) lower average shoot mass and total biomass per plant than individuals in conditions of high nutrient availability. It is suggested that the smaller root systems in low nutrient conditions were not able to achieve the nutrient uptake and therefore growth exhibited by individuals in the high nutrient conditions.

The shoot production in individuals in low water, high nutrient conditions (treatment 2) was similar to or greater than that in individuals in conditions of high water and high nutrient availability. This high shoot production occurred in *D. eriantha* and *E. pallens* despite the fact that these species in the low water conditions did not increase the size of their root systems relative to those in conditions of high water, high nutrient availability. This suggests that compensation for low water availability occurred in treatment 2 individuals. Compensation for low water availability did not however occur when the individuals were also nutrient stressed (treatment 4) since the average plant biomass of these individuals was significantly ($p < 0.05$) lower than that of all the other individuals.

Table 5.3. Differences in root mass:shoot mass ratios between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd, for the five replicates of each treatment are given.

Root:Shoot mass				
Treatment	water	nutrients	$\bar{x}$	sd
<i>A. pubescens</i>				
1	+	+	.80 ^{ab}	.30
2	-	+	.40 ^a	.04
3	+	-	.99 ^b	.14
4	-	-	.66 ^{ab}	.09
<i>D. eriantha</i>				
1	+	+	.47 ^{ab}	.14
2	-	+	.29 ^a	.02
3	+	-	.59 ^b	.06
4	-	-	.52 ^{ab}	.15
<i>E. pallens</i>				
1	+	+	.76 ^a	.17
2	-	+	.77 ^a	.10
3	+	-	.97 ^a	.20
4	-	-	.89 ^a	.08

The root:shoot mass ratios are similarly maintained regardless of treatment. Individuals in treatments 2-4 channelled similar proportions of their reserves into root production as did individuals in high water, high nutrient conditions. Although *A. pubescens* individuals in treatment 2 had a high average root mass relative to that in treatment 1 (Table 5.1), the proportion of the reserves channelled into root production was similar to that in treatment 1 individuals. These results suggest that there may be compensation for low nutrient and low water availability.

A. pubescens and *D. eriantha* individuals in conditions of high water, low nutrient conditions (treatment 3) channelled a higher proportion of their reserves into root production than did individuals in the low water, high nutrient conditions (treatment 2). The average shoot mass in the individuals of these species in treatment 3 was significantly lower than in treatment 2 individuals (Table 5.2). This verifies that increased root production occurred at the expense of shoot production in treatment 3.

Table 5.4. Differences in transpiration rates and in water use efficiencies (WUE) between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd, for the five replicates of each treatment are given.

Treatment	water	nutrients	Transpiration rates (mmolH ₂ O/m ² /s)		WUE (g/mmolH ₂ O)	
			$\bar{x}$	sd	$\bar{x}$	sd
<i>A. pubescens</i>						
1	+	+	1.35 ^a	.40	.16 ^a	.05
2	-	+	.79 ^a	.08	.43 ^b	.04
3	+	-	1.14 ^a	.17	.09 ^a	.01
4	-	-	1.31 ^a	.06	.06 ^a	.02
<i>D. eriantha</i>						
1	+	+	1.07 ^a	.05	.19 ^a	.01
2	-	+	1.03 ^a	.17	.17 ^b	.03
3	+	-	1.41 ^a	.24	.03 ^a	.01
4	-	-	1.29 ^a	.10	.04 ^a	.01
<i>E. pallens</i>						
1	+	+	1.51 ^a	.06	.34 ^a	.01
2	-	+	1.20 ^b	.10	.33 ^a	.08
3	+	-	1.68 ^a	.26	.10 ^b	.03
4	-	-	1.43 ^a	.13	.05 ^b	.01

The analysis of variance test showed that the transpiration rates were similar in all individuals in all the treatments, except *E. pallens* individuals in low water, high nutrient conditions. This indicates that the average water uptake rate must have been similar in individuals in all the treatments. The individuals in low water conditions must have sustained similar water uptake rates to individuals in high water conditions without increasing the size of their root systems. The WUE were significantly ($p < 0.05$) higher in the individuals in the treatments with low water, high nutrient availability (treatment 2), than in all the other individuals, except *E. pallens* individuals, which had similar WUE in treatments 1 and 2.

Table 5.5. Differences in growth rates, between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd, for the five replicates of each treatment are given.

Treatment ^o water nutrients			Growth rate (g dry mass/week)	
			$\bar{x}$	sd
<i>A. pubescens</i>				
1	+	+	.025 ^a	.003
2	-	+	.060 ^b	.001
3	+	-	.009 ^c	.001
4	-	-	.002 ^d	.001
<i>D. eriantha</i>				
1	+	+	.002 ^a	.001
2	-	+	.020 ^b	.001
3	+	-	.001 ^a	.001
4	-	-	.000 ^a	.000
<i>E. pallens</i>				
1	+	+	.125 ^a	.003
2	-	+	.085 ^b	.004
3	+	-	.017 ^c	.002
4	-	-	.000 ^d	.000

Individuals in conditions of low nutrient availability grew significantly ($p < 0.05$) slower during the fourth month than the individuals in conditions of high nutrient availability. These results indicate that no compensation for low nutrient availability occurred. Individuals of *A. pubescens* and *D. eriantha* in the low water, high nutrient conditions grew significantly ($p < 0.05$) faster than all the other individuals of these species. This is also evidence that compensation for low water availability occurred. Little to no growth was detected during the fourth month in individuals in treatment 4 and therefore compensation for low water availability did not occur when the individuals were also nutrient stressed.

Table 5.6. Differences in leaf total nitrogen (N) concentrations between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd, for the five replicates of each treatment are given.

Treatment			N concentration (mg N/g tissue)	
water	nutrients		$\bar{x}$	sd
<i>A. pubescens</i>				
1	+	+	15.22 ^a	.39
2	-	+	15.47 ^a	.02
3	+	-	7.04 ^b	1.39
4	-	-	8.48 ^b	.87
<i>D. eriantha</i>				
1	+	+	14.11 ^a	.71
2	-	+	12.66 ^a	2.61
3	+	-	7.02 ^b	1.49
4	-	-	6.78 ^b	1.08
<i>E. pallens</i>				
1	+	+	20.40 ^a	.55
2	-	+	18.58 ^a	1.70
3	+	-	8.95 ^b	.46
4	-	-	10.28 ^b	2.67

The leaf N concentrations of individuals in the conditions of low N availability were significantly ($p < 0.05$) lower than those of the individuals in the conditions of high N availability. These results show that rhizosheaths do not compensate for low N availability. Individuals which were grown in conditions of high N availability had similar leaf N concentrations despite the two different water conditions. The same trend occurred in the conditions of low N availability. Therefore, low water availability did not affect N uptake by the roots.

Table 5.7. Differences in leaf total phosphorus (P) concentrations between the individuals growing in different conditions of water and nutrient availability. The mean value, $\bar{x}$, and the standard deviation, sd, for the five replicates of each treatment are given.

			P concentration (mg P/g tissue)	
Treatment	water	nutrients	$\bar{x}$	sd
<i>A. pubescens</i>				
1	+	+	1.85 ^a	.23
2	-	+	1.10 ^b	.06
3	+	-	0.87 ^b	.25
4	-	-	0.86 ^b	.11
<i>D. eriantha</i>				
1	+	+	1.91 ^a	.18
2	-	+	1.16 ^b	.12
3	+	-	0.98 ^b	.06
4	-	-	0.64 ^c	.08
<i>E. pallens</i>				
1	+	+	1.60 ^a	.20
2	-	+	1.13 ^b	.40
3	+	-	0.67 ^c	.10
4	-	-	0.52 ^c	.09

The average leaf P concentrations of individuals grown in conditions of low water and/or low P availability were significantly ($p < 0.05$) lower than those of individuals grown in conditions of high availability. These results, as well as those for N, verify that no compensation for low nutrient availability occurred. Under the conditions of high P availability, the individuals in the low water conditions had significantly ($p < 0.05$) lower leaf P concentrations than individuals in the treatments with high water content. Therefore, the low water

availability must affect P uptake by the roots. In the conditions of low P availability the *A. pubescens* and *E. pallens* individuals in the two different conditions of soil water content had similar concentrations of P in the leaf tissue. The *D. eriantha* individuals in the low water conditions had significantly ($p < 0.05$) less leaf P than individuals in high water conditions.

5.4.1 SUMMARY OF THE RESULTS AND THEIR IMPLICATIONS

The plant parameters which were used as indicators of sheath compensation for low water and/or low nutrient availability must vary under different water and nutrient conditions as shown in Table 5.8 if compensation is to be assumed.

Table 5.8 Summary of the levels of the parameters expected when compensation for low water and/or low nutrient availability occurs and when no compensation occurs.

Plant parameter	Compensation	No compensation
	Levels relative to those of plants in high water, high nutrient conditions	
Root mass	similar/low	high
Shoot mass	similar/high	low
Root/shoot mass ratio	similar/low	high
Water uptake rates	similar/high	low
Water use efficiency	similar/high	low
Growth rates	similar/high	low
Nutrient levels	similar/high	low

Table 5.9 summarises the different levels found for the plant parameters. The results may be compared to the expected levels shown in Table 5.8. The "high/low" levels indicate the average levels recorded in the low water, high nutrient treatment (T2), high water, low nutrient treatment (T3) and low water, low nutrient treatment (T4) relative to the levels recorded in the high water, high nutrient conditions (treatment 1). Compensation for low water availability is indicated by the # notation and compensation for low nutrient availability is indicated by the *.

Table 5.9 Summary of the relative levels of the plants parameters measured, indicating compensation (or lack of compensation) for low water (low nutrient) availability.

Plant parameter	Levels relative to those of the plants in treatment 1		
	T2	T3	T4
Root mass	similar/low [#]	low [*]	low ^{*,*}
Shoot mass	high [#]	low	low
Root/shoot mass ratio	similar [#]	similar [*]	similar ^{*,*}
Transpiration rates	similar [#]	-	similar [#]
Water uptake rates	similar [#]	-	similar [#]
Water use efficiency	high [#]	low	low
Growth rates	high [#]	low	low
Leaf N levels	similar ^{*,*}	low	low
Leaf P levels	low	low	low

Compensation for low water availability apparently due to rhizosheath presence, is manifest in eight of the nine measured parameters. Also evident from Table 5.9 is the limited compensation for nutrient availability, but the possible compensation for low nitrogen availability when the low availability is a result of low soil water content (treatment 2) is apparent.

5.5 DISCUSSION

The results of this experiment show that the presence of rhizosheaths compensate for low ~~water~~ availability. This conclusion is reached because the individuals in conditions of low water availability did not increase the production of roots at the expense of shoot production (Table 5.3). While having similar or smaller sized root systems (Table 5.1) these individuals sustained similar transpiration rates and therefore water uptake rates to individuals in high water conditions (Table 5.4). More important than the transpiration rates, in terms of survival and vigour of the plants, is their ability to utilize water efficiently. Although the individuals in the low water, low nutrient conditions apparently took up similar amounts of water, they were not able to utilize the water as efficiently as those individuals which were provided with extra nutrients (Table 5.4). The individuals in conditions with low water availability but high nutrient availability, had the highest water use efficiencies (WUE). *E. pallens* individuals under these conditions may have achieved this high WUE by restricting transpiration (Table 5.4). The individuals in the low water, high nutrient conditions also maintained similar and sometimes greater growth rates to the individuals grown in conditions of high water and high nutrient availability (Table 5.5). However, the compensation for low water availability did not occur when the nutrient availability was also low.

The compensation for low water availability may be a result of the facilitation of easier water extraction and therefore increased water uptake by the roots as a result of the close proximity of epidermal hairs, mucilage, water and soil particles that results from rhizosheath formation.

It is proposed that if the individuals in the low water availability conditions had developed more extensive sheaths, then the compensation would have been even greater and possibly also have occurred in the individuals from the low water, low

nutrient conditions. A greater density of epidermal hairs would occur in a more extensively developed sheath and therefore a larger absorptive surface area would be in close contact with soil water. Unfortunately this was not tested since the only way to alter sheath thickness (within a species, within a set water availability condition) would have been to alter the soil texture and, as explained before, this would have created inherent differences in plant water availability.

Why the *A. pubescens* and *D. eriantha* individuals in the conditions of high water, high nutrient availability (treatment 1) did not have the highest growth rates cannot easily be explained. Possibly during the fourth month, with the substantial increase in extent of sheath development the compensation by the sheaths increased to such an extent that the individuals in treatment 2 were able to grow faster than the individuals in treatment 1, where compensation was not required.

While suggesting compensation for low nutrient availability, the results reject the hypothesis that sheaths compensate for low nutrient availability. The shoot production, growth rates and nitrogen and phosphorus concentrations in the leaves of individuals from low nutrient conditions were lower than in the individuals from high nutrient conditions. The lower root mass recorded for the individuals in conditions of low nutrient availability was a result of lower overall plant growth due to low nutrient uptake rather than compensation due to sheath presence, as initially appeared to be the case. These individuals were stressed and no compensation by the rhizosheaths occurred since the concentrations of N and P taken up by these individuals were significantly lower than those taken up by the individuals in the conditions of high N and P availability. These lower concentrations were not adequate to sustain equivalent growth to those of individuals from high nutrient conditions. Therefore, it appears that the sheaths did not compensate for low nutrient availability.

The N results (Table 5.6) give further evidence to support the theory of sheath compensation for low water availability, and indicate that they may compensate for low N availability when this low availability is a result of low soil water content. In the conditions of low N availability the individuals in the two different soil water conditions had similar concentrations of N in the leaf tissue. The same holds true for individuals in conditions of high N availability. Thus, low water availability did not restrict the uptake of N. This could be because there was sufficient water in the soil of the low water treatments to transport similar amounts of N to the roots in the two different water treatments. Another possibility is that the sheaths increase the movement of water and/or N to (and possibly into) the roots when growing in conditions of low N availability. If the latter is true, then the sheath may compensate for low N availability which results because of low soil water content.

In contrast to the trends found for N, in treatments with high P, individuals of all three species in low water conditions (treatment 2) had significantly lower P concentrations than those in high water conditions (Table 5.7). The presence of sheaths in low water conditions did not influence the rate of water movement to the root surface sufficiently to facilitate similar P uptake rates to those in individuals in high water treatments. Therefore, the lower soil water content restricts P uptake in soils with a high available P content. This is also true for *D. eriantha* individuals in low P conditions.

The results of the leaf N and P concentrations of individuals in the drier soil conditions were different probably because N is a mobile element and P is an immobile element. P is more strongly adsorbed to soil particles than are other nutrients (Singer and Munns 1987) and strong P gradients and diffusion flows are required to move P from the bulk soil to the root surface. In conditions of low water availability P movement to the roots, and therefore P uptake by the roots, is lower than in conditions of high water availability. The presence of sheaths

would influence N movement more than P movement since the amount of any immobile ion brought to the root surface by mass flow is much less than the amount of any mobile ion carried by mass flow. Thicker sheaths may have had more of an effect on P uptake because these would have a higher number of epidermal hairs and therefore a greater absorptive area in contact with soil P. This would increase P uptake, thereby creating a steeper P gradient from the bulk soil to the root surface, thus increasing the rate of P diffusion to the root.

It is concluded that while rhizosheaths present on grass roots do not appear to compensate for low nutrient availability, they do compensate for low water availability. The apparent compensation is evidence to support the predictions of earlier researchers, that sheaths would promote water absorption in arid areas (Price 1911; Henrici 1929 and Marneweck 1990) probably due to the increased production of epidermal hairs (Goodchild and Myers 1987) and the close proximity of these hairs to the mucilage and soil particles. The higher water uptake rates facilitated by the sheaths would increase the rate of water diffusion to the soil-root interface. The high water content at this interface would protect the root hairs from desiccation in arid areas (Price 1911; Marneweck 1990), as well as insulate the roots against moisture loss (Lejstner 1967; Clarkson and Hanson 1980 and Buckley 1982).

This compensation would explain why the average extent of sheath development on sandy soil individuals in low rainfall areas is greater than in high rainfall areas (chapter 3). The individuals in lower rainfall areas require more extensive sheaths in order to take up sufficient water to maintain comparatively high growth rates under these conditions.

As shown in chapter 4, *E. pallens* individuals had more extensive sheath development in all the treatments than both the other species. Corresponding to this, the leaf total N and P concentrations, growth rates and total plant biomass of *E. pallens* individuals in treatments 1-3 were significantly ($p < 0.05$) higher than in both other species. The conditions in treatment 4 are too stressful for detectable growth to occur even in *E. pallens*. The theory proposed in chapter 4 (section 4.4), that *E. pallens* would probably be at a competitive advantage to *A. pubescens* and *D. eriantha*, when growing sympatrically, is substantiated by the results of this experiment. These results show that this would be true particularly in arid environments. This competitive advantage may be directly linked to the presence of thicker rhizosheaths in *E. pallens* when growing under all environmental conditions.

This theory could be tested by simple, controlled growth experiments. These would involve planting two species, each with a distinctly different extent of sheath development, in the same containers. Different sets of these containers could be supplied with different combinations of water and nutrient levels. The plant biomass and growth rates could be used as an indication of which species fared better under each condition.

In order to further investigate the proposed relationship between rhizosheath thickness and influence on P uptake an additional study was conducted. Within-species sheath thickness differences were investigated in relation to P uptake and compensation for low P availability.

CHAPTER 6

THE INFLUENCE OF EXTENT OF RHIZOSHEATH DEVELOPMENT ON THE UPTAKE OF SOIL NUTRIENTS.

6.1 INTRODUCTION

As explained in chapter 5, it was thought that sheaths act to increase the area of soil from which roots are able to extract nutrients, i.e. the zone of depletion. The previous experiment explained that no compensation for low nutrient availability occurred due to sheath presence. A limitation of that experiment was that only the compensation by thin sheaths for low availability could only be studied, since the soil texture could not be varied to stimulate different sheath thicknesses. It was proposed that if individuals in conditions of low nutrient availability developed thicker sheaths then compensation may occur. It was therefore decided to undertake the following experiment in which the extent of sheath development was varied and the effect of thicker sheaths on nutrient uptake studied.

It can be understood from the findings of the study thus far that it is unfortunately not possible to study the within-species differences in root uptake between individuals with thick sheaths and individuals with thin sheaths, unless the individuals are grown in different conditions of soil texture or nutrient and water availability. In this experiment, since nutrient uptake was to be investigated, it was decided to grow individuals in different soil textures in order to induce different extent of sheath development within each species.

The rate of phosphorus (P) movement in most soils is slower than that of other nutrients (Russell 1973). Most of the P taken up

comes from within a fraction of a millimetre of the root surface (Russell 1973). The drier the soil the more difficult the diffusion path for P and the slower the rate of diffusion to the roots. In deficient soils, diffusion and mass flow do not always bring adequate P to the root surfaces to meet plant needs. Supply of P to the plant depends more on the size of the root system, density of its root hairs and intensity of ramifications through the soil than does the supply of any other nutrient (Russell 1973). Rhizosheaths may substitute for an increase in production of roots and increased ramification through the soil by enhancing nutrient uptake (or rather by increasing the effective sphere of nutrient uptake by the roots). This possible influence of the extent of sheath development/sheath thickness on P uptake was investigated.

6.2 HYPOTHESES

It was hypothesised that roots with thick sheaths would extract more phosphorus from a greater volume of soil than roots with thin sheaths, when growing under similar conditions of soil phosphorus levels.

6.3 MATERIALS AND METHODS

6.3.1 Experimental design

Seeds of *Antheophora pubescens* and *Digitaria eriantha* were grown in 50mm diameter containers. Unfortunately seeds of *Eragrostis pallens* were not available at the time of initiation of this study and therefore *E. pallens* could not be included in this study. The containers (a in Figure 6.1) had mesh bases (b in Figure 6.1) which prevented root penetration, but which allowed free water and solute movement through the base.

Half the seeds of each species were grown in soil containing 60% sand and 40% clay such that minimal sheath development occurred (refer to chapter 4, section 4.2). The other half were grown in soil with an 80% sand content, such that extensive sheath development occurred. Forty six containers of seeds per soil treatment were planted for each species. To ensure that the sheath development in the two different soil textures was as desired, plants in two of the pots in each treatment were harvested and the extent of rhizosheath development assessed (Table 6.1) before the experiment was continued.

The soil in all the containers was kept at 70% field capacity by watering with a Long-Ashton solution containing all essential micronutrients and 50ppm nitrogen (KNO_3 and $(\text{NH}_4)_2\text{SO}_4$; 1NO_3^- ; 2NH_4^+). After 10 weeks growth, when the roots had formed a mat (c in Figure 6.1) at the surface of the mesh, another container (d in Figure 6.1) with soil containing 80% sand and a known P concentration, was joined to the base of the initial container (a in Figure 6.1).

The containers (d in Figure 6.1) were placed in a sand tray (e in Figure 6.1) and the water was supplied to the plants via this tray. The plants were not watered from above as this would have resulted in leaching of the P from the top layers of sand in the lower containers. The lower containers also had mesh bases such that free movement of water from the sand tray could occur. Similar soil was used in all the lower containers in order to eliminate differences in the rate of P transport to the roots due to different diffusion coefficients and cation exchange capacities (CEC) of different soils.

During the initial ten weeks of growth no P was supplied to the plants such that when the lower containers were added to the experimental setup the plants were sufficiently P stressed to ensure P uptake by the roots from these containers. The P stress was evident in the slight purple colour in the leaves which the plants started showing towards the end of the ten weeks.

Using three different P concentrations on different plants the effect of the sheath when growing under different concentrations of P availability was investigated. Fourteen replicates\containers per species (between ten and fifteen individuals per container) for each P concentration were used, seven replicates with thick sheaths and seven with thin sheaths. The concentrations used were: 5mg P/kg soil; 115mg P/kg soil, these are the P concentrations at the two extremes of the range found in the different soils in which *D. eriantha* grows naturally; and an intermediate concentration of 50mg P/kg soil. The P concentrations were obtained by adding the correct mass of sodium dihydrogen orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) to the soil for the required P concentration. Sodium dihydrogen orthophosphate was used because it is a primary phosphate and is highly water soluble and the P is therefore readily available for uptake.

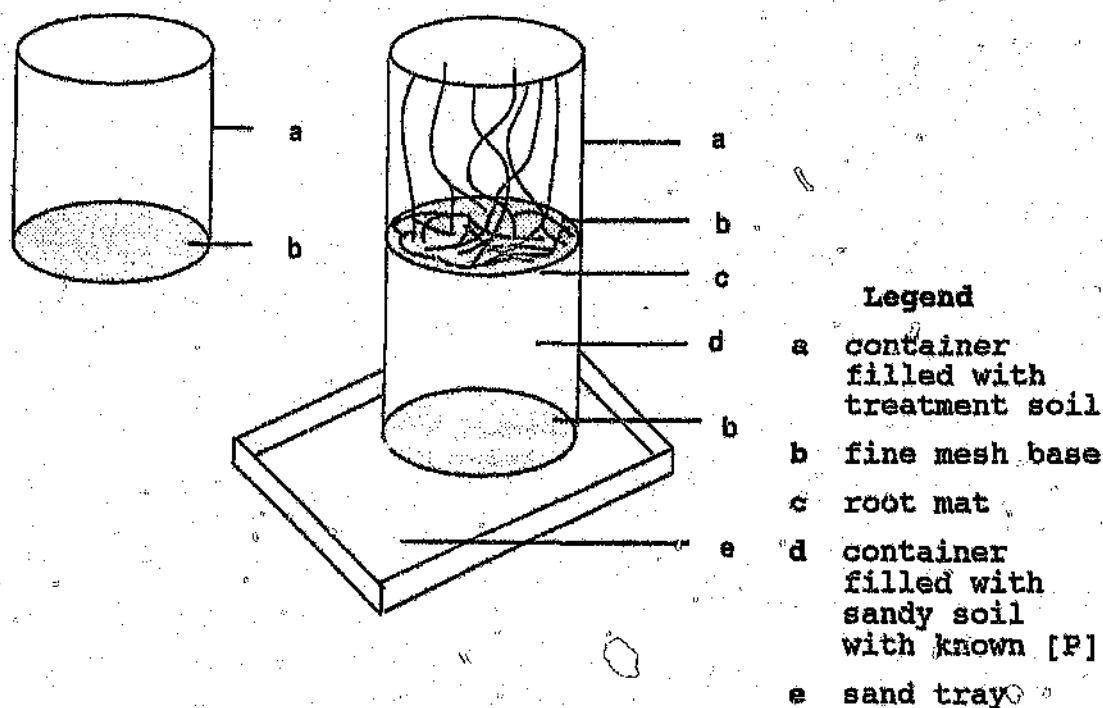


Figure 6.1. Diagrammatic representation of the plant growing technique used (after Gahoonia and Nielsen 1991).

6.3.2 Soil sectioning and analysis.

After 14 days, the lower containers were removed and five consecutive 2mm layers of soil in these containers were sectioned, as shown in Figure 6.2. Thinner sections could not be used since an insufficient amount of soil for the analysis was obtained. After removing the mesh base, the lower container was pushed onto a metal spike on a base plate. A 50mm diameter, 2mm thick disk was then slid under the container, which resulted in 2mm of soil being pushed out of the top of the container. After collecting this soil another 2mm thick disk was slid under the container and the 2mm of soil that extruded from the top of the container collected as the second sample. This was repeated five times such that 10mm of soil was sectioned, since 10mm corresponds to the probable maximum extent of the rhizosphere (section 2.4).

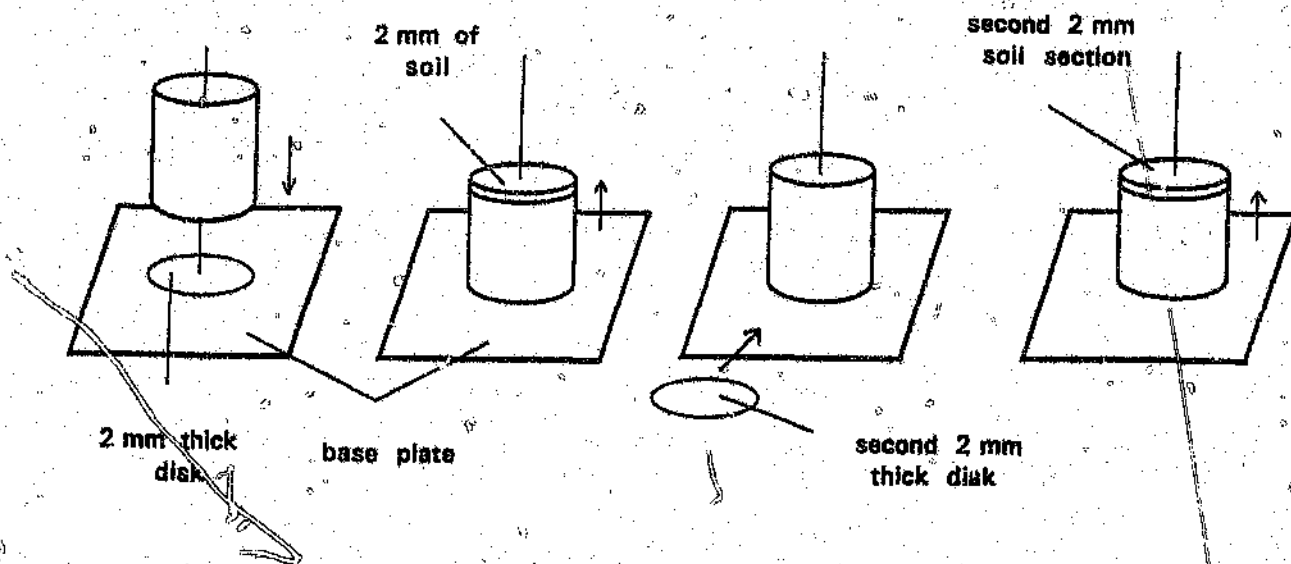


Figure 6.2. Soil sectioning method (after Kuchenbuch and Jungk 1982).

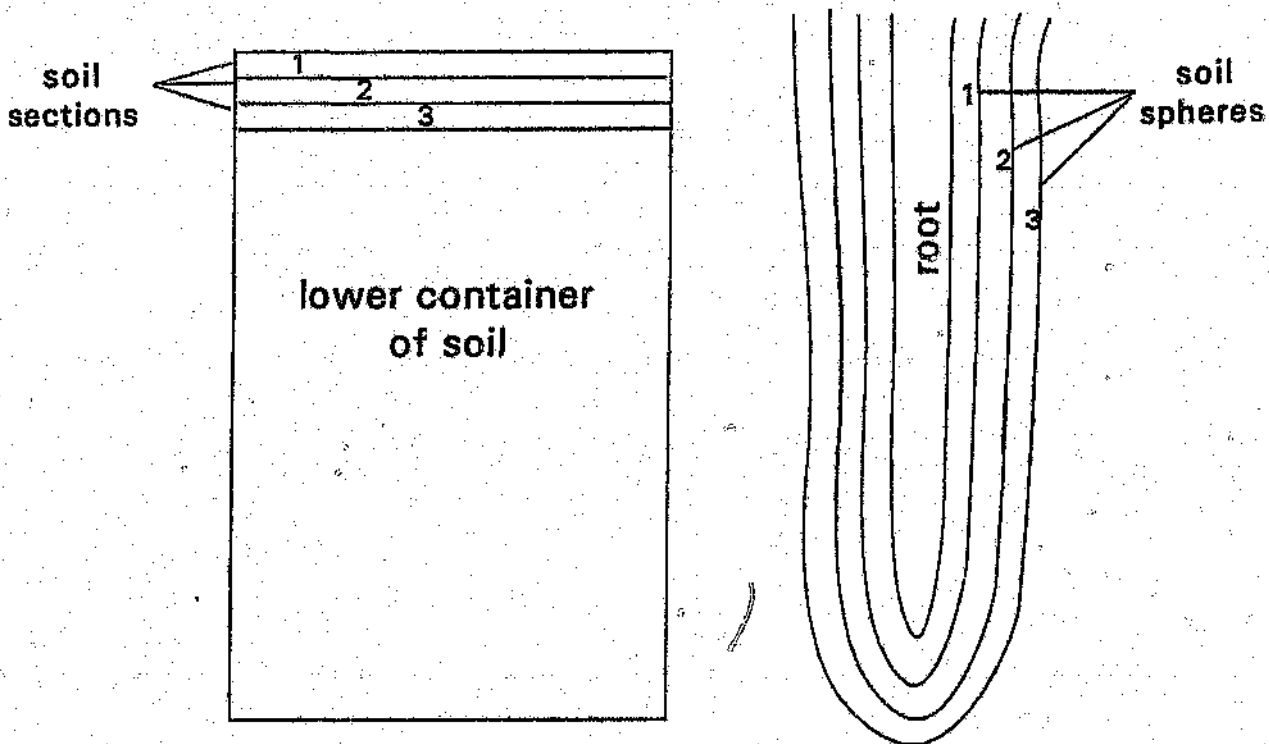


Figure 6.3. Soil sections and the corresponding soil spheres around the root.

The soil sections were analyzed for inorganic phosphate concentration using the resin-bag extractable phosphorus method (Sibbensen 1978). The sections were used as a representation of the soil spheres around the root (Figure 6.3), since it was not practical to study spheres of soil around the roots because the plants had to be grown in soils of different textures. Different textures have different CEC and diffusion coefficients and would therefore have resulted in different rates of movement of P to the roots. This would have resulted in different P uptake rates by the individuals in the different soils, which could not have been attributed to different thickness sheaths.

6.3.3 Statistical analyses.

The effective sphere of influence of thick sheaths on P uptake was investigated by comparing the percentage of the total supplied P that was taken up from each layer of soil by roots with thick sheaths and roots with thin sheaths. Due to the thorough mixing of the soil it was assumed that the added P was evenly distributed throughout the soil.

The data were normally distributed and analysis of variance was used to indicate whether significant variation occurred in the results for each species. Tukey tests were then used to determine significant differences in the percentage uptake of the total supplied P between the thickly sheathed roots and the thinly sheathed roots for each soil section/distance from the roots.

6.4 RESULTS

The desired differences in extent of sheath development (section 6.3.1) did occur between the soil treatments (Table 6.1).

Table 6.1 Differences in extent of sheath development, within the two species, between the two soil textures.

Species	% sand content	Sheath extent
<i>A. pubescens</i>	80	4
	60	3
<i>D. eriantha</i>	80	3
	60	1

Similar results were obtained for both species. At low levels of P availability (5mg P/kg soil) significantly ($p < 0.05$) more P was extracted by thickly sheathed roots, from each soil section, than by thinly sheathed roots (Figure 6.4, a and b). At medium and high levels of P availability (50mg P/kg soil and 115mg P/kg soil respectively) similar amounts of P were taken up from the soil close to the roots (up to 6mm from the root surface), by roots with thick and thin sheaths. However, at distances further from the root, thickly sheathed roots extracted significantly ($p < 0.05$) more P from the soil than thinly sheathed roots.

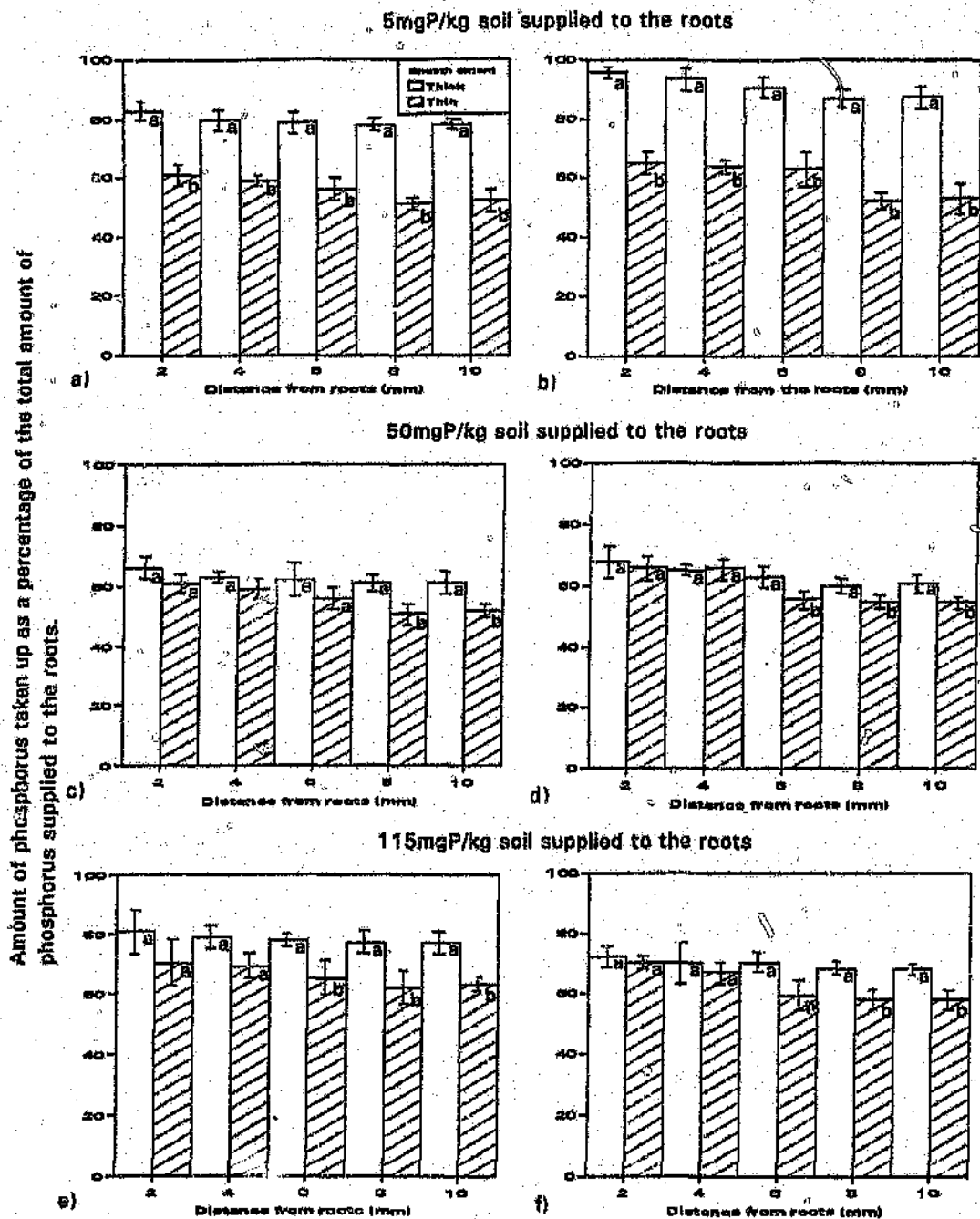


Figure 6.4. Amount of phosphorus (as a percentage of the total amount of phosphorus supplied in the soil) taken up from each soil section by roots of *A. pubescens* (a, c, e) and *D. eriantha* (b, d, f), with sheaths of different thicknesses. The standard deviation bars are shown for each set of results. The different letters on the graphs indicate significant differences between the amounts of phosphorus extracted by the thickly and thinly sheathed roots and only apply within each soil section and to within-species comparisons.

6.5 DISCUSSION.

It is clear from the graphs in Figure 6.4 that sheath thickness has a marked influence on P uptake by roots. The results support the hypothesis since they show that the greater the extent of rhizosheath development the greater the amount of P that is extracted from each soil zone (the more extensive the effective depletion zone).

At low P availability the amount of P extracted from each soil section was significantly greater by thickly sheathed roots than by thinly sheathed roots. This occurred even close to the root surface, where soluble P availability would have been greatest. At medium and high levels of P availability, similar amounts of P were extracted by the thickly and thinly sheathed roots, from soil up to 6mm from the root surface. At distances further from the root the difference in amounts extracted was significant, while being smaller than in low P soils. The difference between the results from the low and higher P availability treatments shows that the presence of thick sheaths is particularly important in soils with a low P status.

In the treatment with the highest levels of supplied P (Figure 6.4, e and f), the thick sheaths may have facilitated luxury uptake (absorption of nutrients in excess of the immediate growth requirements (Chapin 1980)) of P. This is because, judging by the plant vigour in the three treatments, which appeared to be similar, the amount of available P near the root surface in the high P treatment would probably be sufficient for the plant's requirements. The amount of P extracted from the soil at the greater distances from the root would not need to be increased relative to that extracted by the thinly sheathed roots in order to sustain equivalent growth rates and plant vigour.

The results support those of Bhat and Eze (1974 a; b), that the width of the depletion zone around roots increases with increased root hair production. Due to the close proximity of the root

hairs, soil particles, soil solution and nutrients, the rhizosheath environment may facilitate even easier P uptake by the roots. The results in chapter 5 indicate that in the same way the rhizosheath environment facilitates easier water uptake. The thicker the sheath, the more epidermal hairs and the greater the root adsorptive area in close contact with soil nutrients and water. The higher the rate of P absorption from the inner rhizosphere the steeper the P concentration gradient from the bulk soil to the root surface, and therefore the faster available P will diffuse to the root surface from the adjacent soil. Another influence, but of less significance, on P uptake is sheath influence on water uptake. The higher the rate of water uptake the faster water will move to the rhizosphere, bringing with it P from the bulk soil. However, the amount of P carried to the root by mass flow is low compared to the amount which diffuses to the root (Frenzel 1979; Chapin 1980). The thicker the rhizosheath the greater the volume of soil it will influence. Since P is an immobile nutrient, the rate at which it is taken up is limited by the rate at which it moves to the root surface rather than by the rate of transfer into the root. These results clearly highlight the important effect of thick sheaths on root uptake of P (and possibly other immobile nutrients).

It is proposed that if individuals developed thicker sheaths in response to conditions of low nutrient availability (treatments 3 and 4 of the experiment in chapter 5), then compensation for the low availability may occur.

Whether it is possible to genetically manipulate species to develop sheaths is unknown. The possibility of manipulating species to develop thick sheaths when growing under stressful environmental conditions could become an important technique employed in agriculture to increase crop nutrient content, as will be discussed in chapter 8.

The influence of the sheath on P availability may be more complex than initially thought and investigated in this experiment. In addition to facilitating easier absorption of available P, the sheaths may also have an indirect effect on P availability through their influence on rhizosphere microbial populations. The roots affect the rhizosphere environment which affects the microorganisms at the soil-root interface which in turn influence root functioning. Since rhizosheaths are modifications of the rhizosphere environment, they may alter the microbial population in the rhizosphere. The amount of plant available P depends on microbial mineralization of organic P into inorganic P. A higher proportion of P mineralizing microbes may occur in sheath soil relative to that in unaltered rhizosphere soil. This theory will be dealt with in chapter 8 since this experiment considered only plant available P while the following chapter deals with rhizosheath influence on the microbial population.

Another indirect effect on P uptake may result through chemical alteration of the rhizosphere soil due to rhizosheath presence. The most important chemical change, with respect to P uptake, would be a pH change. Different forms of phosphate are taken up by the roots under different soil pH conditions. Plant roots may also increase P solubility in the rhizosphere by releasing certain substances which chelate P sorbents, such as iron and aluminium compounds (Gardner, Barber and Parbery 1983). The microorganisms at the soil-root interface influence root exudation. Any alteration in the microbial population due to sheath presence may alter root exudation and increase the release of substances that chelate P sorbents, thereby increasing P availability.

It has been stated (Singer and Munns 1987) that in order to efficiently extract immobile nutrients, roots must rapidly extend the rhizosphere depletion zone with minimal cost in terms of energy and resources. Since the presence of thick sheaths has now been shown to significantly increase the amount of P extracted from the soil, the development of a rhizosheath must

be a good way of increasing the rhizosphere depletion zone with relatively little cost to the plant in terms of resources for the production of additional plant tissue. This theory will also be discussed further in chapter 8.

CHAPTER 7

THE INFLUENCE OF RHIZOSHEATHS ON THE MICROBIAL BIOMASS AT THE SOIL-ROOT INTERFACE.

7.1 INTRODUCTION.

Plant roots have a direct influence on the composition and density of the soil microbial community (Atlas and Bartha 1987; Richards 1987). The numbers and activities of soil microorganisms decrease with increased distance from the roots and the effect of the roots on the microorganisms decreases proportionally as the distance in space from the root increases (Atlas and Bartha 1987). The microbial growth in the rhizosphere is stimulated by the continual input of readily assimilated organic substrates from the roots, and may also be affected by alteration of the oxygen-carbon dioxide levels, nutrient availability (which in turn may be controlled by acidity), plant species, stage of growth and soil moisture content. Since rhizosheaths are modifications of the rhizosphere and probably alter these conditions, it was proposed that they will affect the rhizosphere microbial population (biomass and composition). Recognition that the biomass of soil microorganisms, namely bacteria and fungi, constitutes a major nutrient source-sink has highlighted the need to quantify the soil microbial biomass and to understand the dynamics of soil populations. It is not easy to quantify the microbial biomass due to their small size and metabolic diversity as well as the complexity of their environment. However, several methods have been devised, involving direct and indirect measurements of microbial biomass (Page, Miller and Keeney 1982).

The direct methods include the cultural method, the microscope method and the most probable number method. In the cultural method the soil sample is incubated on specific media and the

different microbial colonies that develop are counted (Wollum 1982). This method often greatly underestimates the soil population since many cells fail to develop into colonies under the conditions provided. In the microscope methods the microbes from a soil sample are allowed to invade a glass slide or polycarbonate filter and are then counted with the aid of an electron microscope or a light microscope (Schmidt and Paul 1982). The most probable number method permits estimation of population density without an actual count of single cells or colonies (Alexander 1982). This method dilutes the soil samples and measures the microbial growth in each dilution after a certain time.

The direct observation methods described above are time consuming and it is never certain whether all the microbes present have been counted. More easily applicable, non-subjective and replicable methods include the physiological and chemical methods (Parkinson and Paul 1982). In the physiological methods the soil sample is fumigated and then incubated. The flush of carbon dioxide from the fumigated soil which results from the decomposition of microbial cells killed by the fumigation is measured as well as the CO_2 released from unfumigated soil. The microbial biomass in the soil is calculated from the amounts of CO_2 evolved from the fumigated and unfumigated soil and the fraction of the biomass carbon that is mineralized to CO_2 during the fumigation period.

The chemical methods of determining microbial biomass involve the quantitative extraction of a particular compound (for example ATP) found in all components of the microbial community but in no other soil components. High concentrations imply high microbial biomass. All the above methods have shortcomings and it was decided that the best method to use was the indirect method, whereby the microbial carbon (C) and nitrogen (N) concentrations in the soil were chemically determined and used as an index of microbial biomass.

It is often more informative to know the activity of the microbes than their biomass. The activity can be measured by ATP techniques, or indirectly by determining the rate of N mineralization. The ratio of C:N in the substrate may be used as an indication of the microbial biomass but is a better indicator of microbial activity. A high C:N ratio in the substrate indicates a potentially low microbial N mineralization rate. A C:N ratio greater than 20:1 in the substrate indicates net N immobilization (Richards 1987). The ratio of microbial C to N may be used as an indication of the microbial nutritional status. Each class of microorganism has a different C:N ratio and in arable soil these ratios can range from 5:1 (in bacteria) to 10:1 (in fungi) (Miller and Donahue 1990).

In this experiment the effect of rhizosheath formation on the microbial population was investigated. It had previously been noted that the development of a microenvironment which is particularly favourable to microorganisms, in close proximity to grass roots, may be a more important function of rhizosheaths than that of overcoming drought stress (Duell and Peacock 1985).

7.2 HYPOTHESIS

It was hypothesised that the microbial biomass, as indicated by the microbial carbon and nitrogen concentrations, in the rhizosheaths would be significantly higher than that in the rhizosphere and bulk soil.

7.3 MATERIALS AND METHODS.

Within species comparisons of root characteristics and related phenomena (such as rhizospheric microbial populations), between individuals with and without sheaths can not be achieved since when the sheath forming trait is present sheath development always occurs, except in the six species, found during the herbarium study to occasionally not develop sheaths. The conditions under which these species do not develop sheaths is not clear and therefore a manipulative study could not be undertaken to stimulate sheath development and no development within a species. It was decided in this experiment to compare the microbial biomass between species (sheath forming and non-sheath forming species), which grew in close proximity to one another in the same soil type, with the same conditions of water and nutrient availability. The microbial C and N concentrations in the soil around roots of sheath forming and non-sheath forming species were used as an indication of the microbial biomass.

7.3.1 Soil collection.

The soil, surrounding the roots of individuals of three sheath forming grass species and three non-sheath forming grass species growing in a sandy soil environment in a semi-arid savanna in the northern Transvaal, was studied.

SHEATH FORMING SPECIES

Eragrostis pallens Hack.

Pogonarthria squarrosa (Roem. & Schult) Plig.

Schmidtia pappophoroides Steud.

NON-SHEATH FORMING SPECIES

Cymbopogon excavatus (Hochst.) Stapf ex Burt Davy

Hyparrhenia hirta (L.) Stapf

Triraphis andropogonoides (Steud.) Phill.

Unfortunately no *Anthephora pubescens* or *Digitaria eriantha* occurred in the area chosen for this study. This area was chosen because it had a rich grass species diversity.

Soil from three zones around the roots was collected in March 1993. Seven plants per species were randomly chosen. A block of soil 1600mm² around each plant was dug out. Approximately 100g of soil from the following zones was collected and analyzed.

- i) The rhizosheath: soil particles in the rhizosheaths were carefully removed from the roots such that as little plant material as possible was included in the samples.
- ii) The rhizosphere: soil within 0-10mm from the root (non-sheath forming species) or rhizosheath (sheath forming species) surface was carefully collected and as little plant material as possible was included in the samples.
- iii) The bulk soil: this was collected at a distance of 300mm from the outer roots of the plant.

Soil around seven individuals per species was collected. Sub-samples of the soil were analyzed to determine the microbial C and N content in the three soil zones.

7.3.2 Soil analysis.

Chloroform-fumigation-incubation followed by colorimetric methods were used to determine microbial C and N concentrations. The methods in Anderson and Ingram (1993) were altered for use in this study. The chloroform-fumigation-incubation method that was used is applicable to most soils (Tate 1991) and allows realistic measurements of soil microbial biomass (Bonde, Schnurer and Rosswall 1988).

10g of each soil sample were weighed into a centrifuge tube and approximately 25g of each sample were placed into small glass beakers. The samples in the centrifuge tubes constituted the "time zero" samples and those in the beakers the "time one" samples.

The beakers were placed into a desiccator containing a small beaker of chloroform. Chloroform saturated filter paper was positioned around the inside of the desiccator. The desiccator was closed, evacuated and left for 5 days.

After five days the desiccator was opened and 10g of each soil sample were weighed into centrifuge tubes. The following procedure, which had already been done on the "time zero" samples, was then conducted on these "time one" samples.

30mls of 0.5 M potassium sulphate (K_2SO_4) were added to each centrifuge tube. The samples were shaken for 30 minutes then centrifuged at 3000rpm for 10 minutes.

a) Microbial carbon

i) Standard solutions.

To make up the standard solutions 2.377g sucrose were dissolved in 500ml of water, which made a 2mg C/ml stock solution. 0, 2.5ml, 5ml, 10ml, 20ml, and 30ml of the stock solution were pipetted into 100ml volumetric flasks and made up to volume with water. This gave standard solutions of 0, 50, 100, 200, 400 and 600µg C/sample respectively.

ii) Digestion preparation.

10mls of the supernatant of each sample (and standard) were pipetted into a digestion tube. After this, 2mls of sodium dichromate (95g $Na_2Cr_2O_7$ in 2l of water) were added to each digestion tube and the contents were well mixed. 10mls of 95%

sulphuric acid (H_2SO_4) were added carefully to each tube, such that no localized boiling occurred, and the contents of the tubes were well mixed.

All the digestion tubes were then placed into a preheated ($135^\circ C$) digestion block. After 60 minutes the tubes were removed and allowed to cool.

iii) Colorimetric determination

When the samples were cool the absorbances were read at 600nm and the C concentrations estimated from the standard curve.

The microbial C concentrations were calculated by subtracting the "time zero" C concentrations from the "time one" C concentrations.

b) Microbial nitrogen

i) Standard solutions

To make up the standard solutions 4.714g of dry ammonium sulphate were dissolved in 1000ml of water, which made a 1000ug/ml ammonium nitrogen stock solution. 50ml of this solution were pipetted into a 500ml volumetric flask and the solution made up to volume with water. This gave a 100ug/ml N solution. 0, 5, 10, 15, 20 and 25ml of the 100ug/ml N solution were pipetted into 100ml flasks and the contents were made up to volume with 0.5M K_2SO_4 and then mixed well. This gave standard solutions of 0, 5, 10, 15, 20 and 25ug ammonium N/ml respectively.

ii) Digestion preparation

10mls of the supernatant of each sample (and standard) were pipetted into a conical flask. Thereafter 2.5ml of concentrated sulphuric acid were added to each flask to maintain an acidic environment to prevent N volatilization. The contents in each flask was then taken to near dryness on a hot plate in a fume

cupboard.

10ml of sulphuric acid were added (in two 5ml quantities) to each flask and the contents quantitatively transferred into a digestion tube. A very small quantity of selenium catalyst mix was added to each tube. The tubes were then placed into a digestion block and digested at 250°C until the samples were clear.

iii) Colorimetric determination

When the tubes were cool the contents of each tube were made up to 50ml with water.

0.1ml of each standard and sample was pipetted into a test tube. 5ml of reagent N1 (34g sodium salicylate, 25g sodium citrate 25g sodium tartrate and 0.12g sodium nitroprusside in 1l of water) were added to each test tube and the contents were mixed well. After this, 5ml of reagent N2 (30g sodium hydroxide and 10ml sodium hypochlorite in 1l of water) were added to each test tube and the contents were mixed well. After 60 minutes the absorbances were read at 655nm and the N concentrations estimated from the standard curve. The microbial N concentrations were calculated by subtracting the "time zero" N concentrations from the "time one" N concentrations.

7.3.3 Statistical analyses

Data from the three non-sheath forming species were pooled and the data from the three sheath forming species were pooled. The data were normally distributed and therefore non-parametric statistical analyses were not needed. Analysis of variance tests were used to determine any significant variation in the data set. This analysis showed that there was significant variation in the results. Tukey tests were then used to determine between which soil zones the microbial C and N concentrations were significantly different.

7.4 RESULTS

Table 7.1. The differences in microbial carbon and nitrogen concentrations between the sheath soil and rhizosphere and bulk soil zones around the (i) sheath forming species and (ii) non-sheath forming species. The mean value, $\bar{x}$, and the standard deviation, sd, for the seven replicates of each soil zone are given.

Soil zone	Concentrations (ug/g soil)			
	Microbial C		Microbial N	
i sheathed	$\bar{x}$	sd	$\bar{x}$	sd
Sheath	5.52 ^a	0.37	0.25 ^a	0.07
Rhizosphere	3.81 ^b	0.43	0.10 ^b	0.04
Bulk	3.83 ^b	0.58	0.08 ^b	0.04
ii unsheathed				
Rhizosphere	4.05 ^b	0.45	0.11 ^b	0.04
Bulk	2.88 ^b	0.56	0.07 ^b	0.02

Data in the table with different superscripts are significantly ($p < 0.05$) different from each other.

Both the microbial C and the microbial N concentrations in the sheath soil are significantly ($p < 0.05$) higher than those in the other soil zones.

Table 7.2. The differences in the average microbial carbon:nitrogen ratios between the sheath soil and rhizosphere and bulk soil zones around the sheath forming species (i) and non-sheath forming species (ii).

Soil zone	Microbial C:N ratio
i sheathed	
Sheath	16:1 ^a
Rhizosphere	21:1 ^b
Bulk	39:1 ^c
ii unsheathed	
Rhizosphere	26:1 ^d
Bulk	38:1 ^c

Data in the table with different superscripts are significantly ($p < 0.05$) different from each other.

The average C:N ratio in the sheath soil is significantly ($p < 0.05$) lower than in the other soil zones.

Unfortunately, due to the intimate physical association between the root tissue and the sheath soil, unintentional incorporation of root tissue in the sheath soil samples was unavoidable. Incorporation of some root material may also have occasionally occurred in the rhizosphere soil. The damaged roots may release organic C (Norvell and Cary 1992) leading to overestimations of the C content in this soil. Root matter was mostly extracted from the soil prior to analysis. In this study the absolute values are not important, and comparisons were made between the relative concentrations of microbial C in the three soil zones. In addition, the total C concentrations in the soil were not considered and it is assumed that these were similar in each soil zone.

Another confounding effect may be that part of the bulk soil collected around one individual may be part of the rhizosphere of another individual. This could not however be avoided due to the spatial distribution of the plants in the field.

7.5 DISCUSSION

The higher microbial C and N concentrations in the sheath soil relative to the rhizosphere soil of non-sheath forming species indicate that there is a higher number and greater density of microbes at the root surface in sheath forming species than at the root surface of non-sheath forming species. While it has been shown that fungal hyphae do not occur in large numbers in rhizosheaths (Wullstein and Pratt 1981; Buckley 1982 and Goodchild and Myers 1987) it is shown here, in support of the hypothesis, that other microorganisms, mainly bacteria, are present in higher densities in the sheath soil than in other soil zones.

The higher microbial C concentrations in the sheath soil indicate that the roots of sheath forming species may release more assimilable carbon substrates than roots of non-sheath forming species, thus facilitating higher microbial activity in the rhizosheath than in unaltered rhizosphere soil. The sheaths therefore provide an environment more suitable for microbial activity than the other soil zones, including the rhizosphere of non-sheath forming species. There is a positive feedback effect of the larger microbial populations in the sheath since they would stimulate a greater release of substrates from the roots, for example by changing root cell-membrane permeability (Rovira and McDougall 1967; Bowen and Rovira 1976) which would in turn facilitate further microbial colonization and activity in this zone.

The substrates which are released from the roots are the products of photosynthesis. They include amino acids, simple sugars and

polymeric carbohydrates, all of which have a narrow C:N ratio and are easily decomposed. The utilization of these substrates by microorganisms results in secondary productivity and recycling of the nutrients. Therefore, these microorganisms can in turn influence the plant as a primary producer (Lynch and Whipps 1990) through their influence on the availability of plant nutrients such as nitrogen.

Due to the compaction of the soil particles in the rhizosheath, the diffusion of soluble carbon products from the soil-root interface to the bulk soil may be restricted by the presence of a sheath. The soluble carbon products would therefore concentrate in the rhizosheath soil, thus providing more substrate for the microbes and facilitating increased microbial colonization, activity and thus N mineralization. Diffusion of these products from the surface of non-sheathed roots would be easier since the spaces between the soil particles are larger, allowing freer movement.

The carbon compounds in the bulk soil have a higher C:N ratio, and are more resistant to decomposition and the overall microbial activity is lower than in the substrates in the rhizosheath or rhizosphere. The compounds in the bulk soil include cellulose and lignin. The recycling of this carbon and the mineralization of the N is slower than in the rhizosheath and less N is released per unit of C than in the rhizosheath.

The high microbial N concentration in the sheath may indicate that there is a high concentration of N incorporated in the microbes, and therefore unavailable for root uptake. This would suggest that the large microbial population is not beneficial to the plant. However, although the microbes initially immobilize the N, as the microbial population turns over, N is released and becomes available for uptake by the roots. The rates of soil N mineralization and immobilization must be understood since the availability of N is the result of these two opposing processes.

The C:N ratios in the microorganisms differ between the three soil zones (Table 7.2). All the ratios are higher than the range recorded in microbes in arable soil (5:1 - 10:1) (Miller and Donahue 1990). This is a reflection of the low N status of the soil in the study site since the amount of C and N immobilized within the microbial population is related to the soil N levels (Wardle 1992). The microbial C:N ratio in the sheath microbes is lower than in the other zones which suggests that as this microbial population turns over, more N will be released to the soil per unit of C, than will be released in the other soil zones. The microbial C:N may also be indicative of the ease with which the substrate is decomposed, the lower the C:N, the higher the potential N mineralization rate (Richards 1987). Therefore, as expected, the C:N ratio results indicate that the substrates in the sheath are more readily decomposed than those in the other soil zones.

The conditions in the rhizosheath, such as the high soluble C availability and the high water content, may be such that decomposition rates are increased, resulting in rapid turnover of the microbial population and rapid N release to the soil. The higher the microbial density, the higher the decomposition rate, provided the environmental conditions are suitable (moist, warm soil and small particle sizes (Miller and Donahue 1990)), the higher the mineralization rate and the greater the overall N availability.

An important point to note is that the cyclic turnover through organic and inorganic forms, mediated by the microorganisms, minimises losses through leaching and provides a continual supply of ions at the root surface. It is therefore concluded that the high concentration of microorganisms at the soil-root interface of sheathed roots is beneficial to the plant.

The results of this study and those in chapter 6 indicate that if the individuals in the experiment in chapter 5 had developed thicker sheaths when growing in conditions of low nutrient

availability, then the concentrations of N taken up would have been greater than those which were recorded. The thicker the sheaths the larger the suitable environment for microbial activity and the higher the rate of N mineralization. The higher concentrations of available N at the soil-root interface would probably have resulted in higher concentrations of N being taken up by the roots. A limitation of the experiment in chapter 5 was that only compensation by thin sheaths for low nutrient availability could be studied, for reasons explained earlier.

A higher concentration of microbial N does not necessarily mean that a higher proportion of the total soil N is immobilized within the microbes and therefore unavailable to the roots. The higher microbial N concentrations may indicate a higher concentration of total N in the soil. Therefore, although a higher concentration of N is incorporated in the sheath microbes, this may be as a result of a higher amount of total N present in the sheath soil relative to that in the other soil zones.

It must be remembered that, rather than the density of the microbes, the composition of the microbial population and relative proportions of the different types of microbes is more important in determining whether a high microbial concentration at the soil-root interface is beneficial to the plant. Some microorganisms may exist in low numbers but may have a considerable influence on the soil biological processes such as N mineralization. The theory that rhizosheaths affect microbial population, initially proposed in chapter 6, is supported by the results of this experiment and will be elaborated on in chapter 8.

Rovira (1979) stated that the ultimate goal in studies of the biology of the soil-root interface must be the manipulation of microorganisms in this zone to increase plant growth. He also indicated that there was a lack of information relating to this. The results of this study indicate that the presence of rhizosheaths on grass roots alters the microbial density and/or

possibly the microbial composition at the soil-root interface. If this is true and if it is possible to genetically manipulate crop plants to develop uniformly thick sheaths under stressful environmental conditions, then the microbial population at the soil-root interface of these roots may be favourably altered such that plant growth would be enhanced.

Further studies are required to determine the relative proportions of functionally different microbes in the sheaths. The microbial population includes bacteria, Actinomycetes and fungi, each of which are responsible for the recycling of a different range of elements. The actual microbial composition of rhizosheaths must be investigated and compared to that in the rhizosphere soil of unsheathed roots. The manipulation of microorganisms at the soil-root interface is possibly an important technique which could be employed to enhance crop growth, as well as P, N and protein content. This will be discussed in more detail in chapter 8. The composition of the microorganism population in this zone must first be determined before it can be manipulated.

PART IV

SUMMARY AND OVERALL CONCLUSIONS

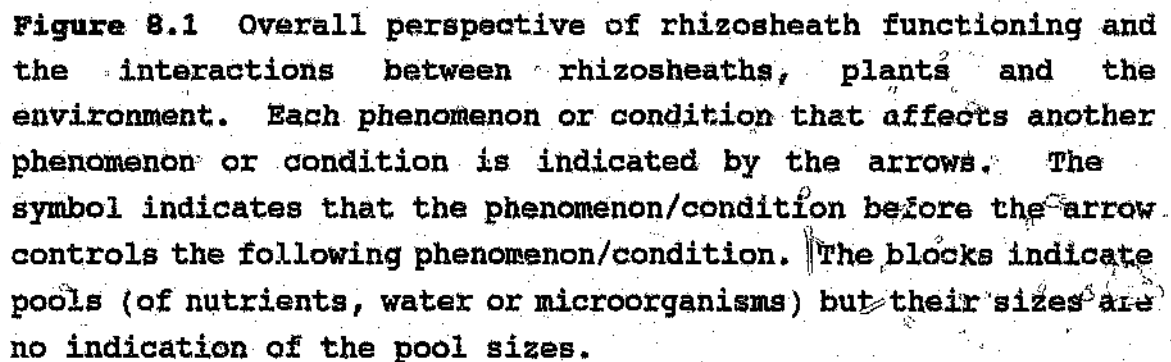
CHAPTER 8

OVERALL PERSPECTIVE OF RHIZOSHEATH OCCURRENCE AND FUNCTIONING

8.1 INTRODUCTION

This study generated unique information on rhizosheaths much of which is fundamental to any future studies, and relevant to a number of aspects in ecological studies and agricultural biotechnology. The distribution of rhizosheaths among grass species indigenous to South Africa and the distribution of the sheath forming species in relation to soil and rainfall conditions has been shown. The study also highlights a fundamental difference between the presence of rhizosheaths and the extent to which they develop. Insight into rhizosheath functioning has also been gained from this experimental work.

The proposed system of rhizosheath development and functioning, as well as the feedback effects between soil properties and composition, and the rhizosheath, is shown in Figure 8.1. This conceptual framework will be used as the basis for discussing the results of this study. The letters in parentheses in the text correspond to those in Figure 8.1.



Rhizosheaths are sandy coatings covering the entire length of each root and are found on the fibrous root systems of many grass species. They are a genetically fixed trait (A) occurring in more than ninety five percent of the grass species in South Africa. However, less than five percent of the species have consistently thick, well developed sheaths when growing under all environmental conditions. The extent to which the sheaths develop, i.e. the thickness of the sheaths and the degree to which the sand particles are held in the sheath, varies between, and sometimes within, species.

The sheaths consist of a mass of sand and silica particles matted together in mucigel by a meshwork of prolific epidermal hairs. The epidermal hairs provide mechanical binding (Wullstein and Pratt 1981) (B), the main factor responsible for the strength of the sheath. The mucigel around the root is important for consolidation of the sheath. The mucigel consists of substances released by the roots (C1 and C2) (Wullstein and Pratt 1981; Guinel and McCully 1987) and from the microbes in the rhizosphere (Rovira *et al* 1979) (C3). The release of substrates from the roots enhances the colonization of the sheath by microbes (D), which require the organic carbon substrates for their metabolism. These microbes at the root surface often enhance root exudation of substrates (E), for example by altering the permeability of the root cells (Rovira and McDougall 1967; Bowen and Rovira 1976). Sheath forming species have been shown (Henrici 1929) to be particularly prone to cortical cell disintegration, especially when exposed to dry conditions. Therefore a large amount of substances are released by the roots of these species, regardless of the microbial affect on root exudation. The enhanced release of organic carbon products in turn stimulates microbial activity. The more products there are available in the rhizosphere for microbial decomposition, the greater the colonization by microbes (D), which leads to a greater release of mucigel substances in the rhizosphere. The more mucigel the more sand particles which can be incorporated into the sheath and the thicker, more consolidated the rhizosheath. The thicker the sheath the higher

the number of microbes which can colonize the sheath. Although bacteria are prolific in the sheath, fungal hyphae do not occur in large numbers (Buckley 1982; Goodchild and Myers 1987 and Wullstein and Pratt 1981) and therefore do not contribute to sheath formation.

The sheaths may extend up to 3mm from the root surface. They are therefore modifications of the rhizosphere (Atlas and Bartha 1987) or part thereof, since the rhizosphere generally extends from the root surface to between 1mm and 10mm from the surface (Jenny and Grossenbacher 1963).

Many root features characteristically vary with the presence of rhizosheaths and extent of their development. The more epidermal hairs that are produced the more extensive the rhizosheath development (B). The more extensive the sheath development the lower the degree of root branching and the smaller the root system (F). This is because sheaths compensate for low water availability (K1 and K2) and low phosphorus availability (L1 and L2). Extensive sheath development probably lowers the need for increased root production, by maintaining sufficient water and nutrient uptake (Y).

8.2 RHIZOSHEATH OCCURRENCE AND FUNCTIONING

While the ability to develop rhizosheaths is a genetically fixed trait, the extent to which the sheaths develop is also a genetically fixed trait within many of these species. The extent of sheath development in these species is uniform between all individuals within a species, although it differs between species. Within the rest of the sheath forming species the extent to which the sheaths develop is a facultative response to environmental conditions (G). The condition which has the most marked influence on the extent of development is soil texture.

Many species in which the sheath forming trait is present do not have restricted distribution ranges. As predicted by Marneweck (1990) and Duell and Peacock (1985), rhizosheaths are geographically ubiquitous (within South Africa), since sheathed individuals are found in all soil types and also in all rainfall regions. However, although extensive sheath development is not exclusive to individuals in sandy soil, a relationship between extent of development and soil texture is evident. This is because individuals growing in sandy soil often have more extensive sheath development than individuals of the same species growing in clayey soil.

It is proposed that there may be a response, in terms of an increased extent of sheath development, to increased sand content in the soil. Sandy soil has a lower water retention capacity (Hillel 1971), lower cation exchange capacity and hence a greater degree of nutrient leaching than clay soil (Singer and Munns 1987). Sandy soil therefore has a lower water and nutrient content than clayey soil (H). Thicker sheaths in sandy soil appear to compensate, as predicted by Goodchild and Myers (1987), for low availability of water and nutrients, particularly P, by facilitating increased water and nutrient movement to the root surface.

A relationship between extent of development and rainfall conditions also exists, with the average extent of sheath development on sandy soil individuals in arid and semi-arid areas being greater than that in higher rainfall areas. This is possibly because the sheaths compensate for low water availability, as predicted by Price (1911), Henrici (1929), Goodchild and Myers (1987) and Marneweck (1990). More extensive sheath development in low rainfall areas may maintain water uptake rates by roots (I), and consequently growth rates (J), similar to those in individuals growing in high rainfall areas.

The compensation for low water availability probably occurs due to facilitation of water extraction by the roots as a result of the close proximity of epidermal hairs, mucilage, water and soil particles (K1). It is hypothesised that the more extensive the development of the sheath the greater the compensation would be (K2), since a greater density of epidermal hairs would occur and therefore a larger absorptive surface area would be in close contact with soil water. Increased water uptake rates have implications for nutrient uptake (L2). Phosphorus, being an immobile ion, is restricted more by its rate of movement to the root surface, than by its transfer into the root (Russell 1973). The thicker the sheaths the greater the effect on phosphorus uptake. The enhanced water movement into the roots, and therefore to the root surface, would increase phosphorus movement to the roots. Since mass flow only provides a small percent of the total phosphorus requirement of the plant, phosphorus diffusion to the root is more important (Prenzel 1979; Chapin 1980). Therefore, more influential with regard to enhanced phosphorus uptake is the close proximity of the absorbing surface (root hairs) and the soil phosphorus. This intimate relationship facilitates phosphorus uptake (I2). The faster the uptake the steeper the phosphorus concentration gradient from the bulk soil to the root surface and the faster phosphorus will diffuse to the root. It has been shown that enhanced phosphorus uptake, due to the presence of thick sheaths, is particularly influential in plants growing in soils with low phosphorus availability. The higher phosphorus uptake enhances plant phosphorus and nitrogen nutritional status, plant biomass production and the overall plant growth rates (J).

Since thickly sheathed roots extract more soil phosphorus than thinly sheathed roots, the phosphorus concentrations will be higher in the thickly sheathed plants. Consequently, levels of substances such as phospholipids, nucleic acids, coenzymes, ATP and ADP will be higher in these plants. It is hypothesised that, besides increasing the phosphorus nutritional value of the plants, the higher levels of phosphorus compounds, such as ATP,

will result in a higher nitrogen content in the crop. The high ATP levels will facilitate greater uptake of nitrate (NO_3^-), which is taken into roots by active uptake mechanisms requiring the energy liberated by the hydrolysis of ATP to ADP (Haynes 1990). The increased nitrogen content in the plant would increase amino acid synthesis and hence the overall protein content of the plants (U).

8.3 LARGE SCALE EFFECTS OF RHIZOSHEATHS.

The importance of rhizosheaths must not be underestimated. Their influence is not restricted to the individual plant, but probably has large scale effects in terms of species distribution ranges, soil microbial activity and prevention of soil erosion.

8.3.1 Distribution ranges

The apparent compensation for low water and nutrient availability suggests a method whereby the sheath forming species are suited for survival (S) in low rainfall regions and in nutrient poor (eg. sandy) soils. The non-sheath forming species which exist under these conditions must either tolerate the low availability of water/nutrients or compensate for it by some other means of extending the rhizosphere depletion zone, for example by increasing the size of the root system.

The development of thick sheaths must be a cost effective way of increasing (widening) the nutrient and water depletion zone relative to other methods employed by plants to deal with stressful conditions. Under stressful conditions the carbon allocation pattern in plants changes and more carbon is channelled to below-ground plant parts (Chapin 1980), both for the increased production of epidermal hairs and for increased root exudates. Species without rhizosheath development would probably increase the carbon allocation into root production to an even larger degree, since not only root hairs but also roots,

must be produced in order to maintain water/nutrient uptake rates. Therefore, sheath development is probably a cost effective mechanism for tolerating arid, low nutrient conditions and avoiding water or nutrient deficiency. The production of more roots to extend the depletion zone is more expensive in terms of resources, since they require production of protective, absorptive and conductive tissue, in addition to epidermal hairs. It is possible that sheathed roots, being more resistant to adverse conditions, such as dry soil and soil compression (Thomas 1922; Oppenheimer 1960), may survive longer than the fine roots of non-sheath forming species. These fine roots, being unprotected, would be more vulnerable under these conditions. Although sheath development requires release of more carbon products to the soil and extensive root hair production, the continual production of many fine unsheathed roots may be more expensive to the plant in terms of resources, than the fewer, less frequently produced sheathed roots. The presence of rhizosheaths may also be less expensive to the roots than the formation of mycorrhizal associations, since many nutrients are absorbed from the host plant by the mycorrhizae (Atlas and Bartha 1987).

The development of rhizosheaths occurs as the root develops, facilitating uptake of water and nutrients from a larger soil volume as the root extends through the soil. Therefore, an increase in the width as well as the extent of the depletion zone is simultaneously accomplished in sheath forming plants.

8.3.2 Microbial activity

As has been discussed, the microbial populations in the rhizosphere influence the extent of sheath development (C3 and E). The relationship between roots and microbes contributes to rhizosheath formation. It appears that the rhizosheath creates an environment more suitable for microbial activity than the rhizosphere environment around an unsheathed root, since the microbial biomass in the rhizosheath is significantly higher than

in the rhizosphere as well as the bulk soil. It is hypothesised that since rhizosheaths are modifications of the rhizosphere, the formation of a rhizosheath affects the composition of the microbial population at the soil-root interface due to the altered rhizosphere environment. The results of the study done by Wullstein et al (1979) support this theory of sheath alteration of the rhizosphere microbial composition. Their results showed that increased nitrogen fixation was associated with rhizosheaths, thus indicating a higher number of active nitrogen fixing bacteria in the sheath soil relative to that in unaltered rhizosphere soil.

The size, as well as the composition, of the microbial population in the rhizosheath influences the overall activity occurring in the sheath (P). A high proportion of the microbial population at the rhizosheath soil-root interface may be constituted by those microbes which are responsible for nitrogen mineralization/recycling (M) and phosphate mineralization/solubilization (N). This high density of microbes in the rhizosheath may result in a higher concentration of plant available nitrogen and phosphorus at the soil-root interface than at this interface of unsheathed roots in a similar environment. The higher availability facilitates greater uptake of these nutrients (I2).

Increased uptake of phosphorus (I2) by thickly sheathed roots relative to thinly sheathed roots has been shown conclusively in this study. It is attributed to the enhanced uptake due to the close proximity of the epidermal hairs, soil solution and soluble phosphorus and to the increased P diffusion, and to a lesser degree water movement (and thus phosphorus movement), to the roots. However, the influence of sheaths on the overall availability of phosphorus must not be overlooked. Through sheath influence on microorganisms (O), soluble phosphorus concentrations may be affected since certain microbial species solubilize phosphate from compounds of low solubility and thereby increase plant available phosphorus (Russell 1973; Richards 1987)

(N). Assuming that the sheaths do increase the proportions and density of favourable microorganisms, the rhizosheaths could contain higher concentrations of the phosphorus solubilizing species of microorganisms than those species which compete with the roots for phosphorus.

The effect of rhizosheaths on soil microbial populations may be influential in the overall below-ground productivity (Q) of any system rich in grass species. Both primary (root) (R) and secondary (microorganisms) (Q) below-ground productivity will be affected. This hypothesis is proposed since, as explained earlier, it is thought that the sheaths may change the composition of the microbial population through their affect on the soil environmental conditions. The microbial activity in turn affects root productivity and therefore root uptake (V). The increased below-ground productivity would be reflected in increased above-ground plant growth (T) and overall plant vigour (S).

If this is true and if it is possible to genetically manipulate crop plants such that the trait for the development of thick sheaths could be fixed in the genome, this would have significant importance in agriculture. The P, N, and therefore protein, content of crops could be increased through the manipulation of rhizosheath development. This would be particularly relevant in crops growing in nutrient poor soils and in semi-arid to arid areas in South Africa. The influence on the microbial population would be of particular importance in the low nutrient status soils since in addition to affecting the availability of nitrogen and phosphorus through recycling and solubilizing, the microorganisms also minimise losses through leaching (W) by temporarily immobilizing the nutrient ions within their bodies. Therefore, the microbial utilization of the nutrients affects the overall availability of the ions at the soil-root interface (X).

8.3.3 Soil erosion

It has been recognised that grass plants are especially good ground cover plants for the prevention of soil erosion. Species which have extensive sheath development, for example *Stipagrostis namaquensis* (Table 3.1), have been recognised as useful soil binding plants and have been used to control soil erosion in overgrazed areas (Gibbs Russell, Watson, Koekemoer, Smook, Barker, Anderson and Dallwitz 1990). The ability to prevent soil erosion has however only been attributed to the shallowness of their fibrous root systems and the degree with which the roots cling to soil particles. The phenomenon of rhizosheath formation and its relevance to soil binding through modification of the rhizosphere soil has not been adequately recognised in terms of its contribution to the prevention of soil erosion. The root systems in which sheaths occur are fibrous, lateral spreading systems and therefore much of the soil in the top horizon may be bound into rhizosheaths rendering it less susceptible to erosion forces.

Grasslands rich in thick-rhizosheath forming species may be less prone to soil erosion losses due to the additional binding effect that rhizosheath formation has on the soil. This makes these species particularly useful in revegetation and soil erosion prevention projects.

8.5 CONCLUSIONS

Rhizosheaths occur in the majority of grass species indigenous to South Africa. The distribution of sheath forming individuals is not restricted to sandy soil nor semi-arid to arid areas. The extent of sheath development within some species varies depending on the soil conditions. The higher the sand content in the soil the greater the extent of sheath development. The difference in extent of sheath development is a direct response to the sand content in the soil, and not in response to the low water or low nutrient conditions of the sandy soil. However, the presence of rhizosheaths appears to compensate for low water availability and thick sheaths increase phosphorus uptake from soil, especially when the phosphorus content is low. In addition to this direct influence on phosphorus uptake, rhizosheaths indirectly influence nutrient uptake. The sheaths affect the microbes and their activities adjacent to the root and thereby influence phosphorus and nitrogen availability at the soil-root interface.

The presence of rhizosheaths, especially thick sheaths, not only substantially influences plant growth and survival in stressful conditions but thick sheaths also enhance the phosphorus, nitrogen and probably the protein content of the tissue.

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