AN INVESTIGATION OF THE LIMITED DISTRIBUTION OF GRAZING LAWNS IN HLUHLUWE-UMFOLOZI PARK, SOUTH AFRICA

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Abstract

The grasslands and savannas of the Hluhluwe-Umfolozi Park (HUP), South Africa contain unusual 'grazing lawn' communities dominated by short, grazing-tolerant grasses. These grazing lawns appear to be highly productive, and support high densities of grazers during the late summer and early winter, but have a very limited distribution within HUP. A review of the grazing literature indicates a similar pattern at the global scale, with grazing lawns absent or rare in almost every grass-grazer system described. Resource limitations responsible for the limited distribution of grazing lawns were investigated. A conceptual model of key processes considered necessary for the formation and persistence of lawns in HUP was developed. Key processes include both frequent and non-selective grazing during the growing season. Based on observations that the distribution of grazing lawns within HUP is strongly related to rainfall, it was hypothesized that further key processes control are involved. In areas of lowest rainfall grazing lawns are replaced by a sparse cover of forbs and unpalatable grass species. It was hypothesized that a shortage of mineral nitrogen (N) and / or water are key processes excluding grazing lawn grasses in these environments. In areas of highest rainfall grazing lawns do not form, even where grass communities have been heavily grazed for a year or more. It was hypothesized that below-ground competition for N by the dominant grasses (bunch grasses) prevent the establishment of grazing lawn grasses in these environments.

To test for the effect of competition by bunch grasses in the high rainfall parts of HUP, a simple transplant and removal experiment was performed by clearing small plots with herbicide. Two species of lawn grass common at lower altitude, *Digitaria longiflora* and *Dactyloctenium australe,* were transplanted in both cleared and control plots (dominated by bunch grasses). Massive differences in the biomass of transplants at the end of one growing season indicated the strong competitive effect of bunch grasses on lawn grasses.

To test the hypothesis that a shortage of mineral N (resulting from bunch grass growth in mesic environments, and low soil moisture in more arid environments) prevents the formation of grazing lawns, N requirements of common lawn grasses were determined with a series of pot experiments. Growth responses to source of mineral N were tested by growing three common lawn species, *D. longiflora*, *D. australe* and *Urochloa mosasmbicensis* in sand culture, with N supplied as either nitrate, ammonium or ammonium nitrate. No significant differences were found in terms of relative growth rates (RGR) or allocation to roots (root mass fraction). Growth responses to rate of N supply were tested with a series of solution culture experiments, using four lawn species, *D. longiflora*, *D. australe, U. mosasmbicensis* and *Sporobolus nitens*. Individuals of each species were grown on a range of ammonium nitrate concentrations. The hypothesized result, of a common, strong growth response to N concentration, was not observed. Instead the four species showed a range of growth responses, with significant differences found for response curves fitted to RGR data for five of six pairwise species comparisons. A similar range of responses was found for various biomass allocation variables, including root mass fraction, leaf area ratio and specific leaf area.

Field fertilization experiments using two of these lawn grasses (*D. longiflora* and *D. australe*) transplanted to a high rainfall site in HUP revealed similar patterns. A factorial experiment with 5 levels of ammonium nitrate addition and shading was conducted over the course of a single growing season. Shading was manipulated by tying up the canopies of bunch grass surrounding transplanted lawn grasses. In this way, light availability was shown to be a significant factor affecting the growth of both species. However, fertilization only produced significant (and weak) increases in growth (in terms of final biomass) for *D. australe*, and only when shading was reduced. A similar factorial experiment was conducted concurrently at a semi-arid site, to test the responses of *U. mosasmbicensis* to N and water. Water addition (by means of irrigation at a range of time intervals) caused a significant decrease in the basal cover of a naturally occurring population of this grazing lawn grass. This was attributed to bunch grass competition resulting from the above-average rainfall during previous and current year of the experiment. N fertilization had no significant effects.

Two final field experiments were conducted to test for the presence of any form of competition from bunch grasses at a heavily-grazed, high rainfall site. Plastic-lined root trenches were dug on either side of emerging stolons of *D. longiflora* and *D.*

australe and all bunch grasses removed from between each pair of trenches. In addition all bunch grasses adjacent to the trenches around half these target stolons were removed, to eliminate any potential shading. Both the stolons and the crowns from which they were growing were measured at regular intervals over the course of a growing season. Root trenching, alone or together with the clearing of adjacent bunch grasses, produced no significant differences in a range of growth response variables (frequency of flowering, rate of shoot production, rate of stolon elongation, frequency of stolon nodal rooting and stolon longevity). In the second experiment, conducted concurrently, the same growth variables were measured on a number of long-established grazing lawns at lower altitude sites. Again, no significant differences were found.

From the results of the above experiments, it was tentatively concluded that a high supply of N, or any other below-ground resource, is not a key process limiting the distribution of grazing lawns. Water was still considered a likely limiting factor in semi-arid environments. For mesic environments, it was speculated that the distribution of grazing lawns is instead restricted by a multitude of limiting resources (each relevant to different lawn species), or that a shortage of frequent, non-selective grazing alone is the only key process involved.

CHAPTER 1

INTRODUCTION

This thesis investigates the co-existence of different plant species under conditions of intense herbivory. The overall aim is to establish the environmental conditions necessary for grazing-tolerant grasses to compete with taller, grazing-intolerant grasses in ecosystems containing ungulate grazers. Investigations focus on the resource requirements of the grazing-tolerant species. The specific context of the study is a game reserve, the Hluhluwe-Umfolozi Park (HUP) which supports an abundance of large herbivores as well as patches of communities of grazing-tolerant grasses, referred to as **lawn grasses**. These lawn grasses have a markedly different growth form to neighbouring grasses and the communities they dominate, **grazing lawns,** tolerate a high level of grazing, and support high densities of grazers in the summer. Questions posed in the thesis centre around the limited distribution of these grazing lawn communities, both in HUP and world-wide, and are of relevance to the management of all ecosystems which do, or did, support large densities of grazers.

This chapter consists of two sections. The first describes grazing lawn community patterns, in HUP, and world-wide. The second establishes a conceptual framework for investigating the limited distribution of grazing lawns, both in HUP and in grass-grazer systems in general, and introduces the main hypotheses which were tested.

THE GRAZING LAWN PATTERN

Specific context - grazing lawn patterns in HUP

Study site

The Hluhluwe-Umfolozi Park (**HUP**), KwaZulu-Natal, South Africa, is a fenced reserve of just over 900km^2, containing forest, savanna and grassland vegetation communities. The topography of the park is hilly, with undulating lowlands in the eastern and south-eastern parts and steeper uplands in the north and west (**Fig. 1.1**). The overall altitudinal range is 540m. The entire park has a coastally modified climate with mean annual rainfall ranging from 990mm at high altitude down to under 635 mm at low altitude. Mean minimum temperature is 13EC, mean maximum temperature is 35EC, and frost is rare (Balfour and Howison 2002). The soils of the park are generally eutrophic being largely derived from shales and sandstones intermittently fractured by doleritic intrusions (Balfour and Howison 2002). Brookes and MacDonald (1983) provide a general description of the common fauna and flora in the park, as well as previous changes in large herbivore numbers and management actions.

Herbivore numbers are unusually high for a southern African game reserve – ungulate densities peaked at about 89 kg ha^{-1} in the Umfolozi section of HUP in 1970's (Owen-Smith and Danckwerts 1997), higher than other southern African game reserve and common livestock stocking rates. In addition, Owen-Smith and Danckwerts estimated grass consumption in the Umfolozi section to be 544 kg ha^{-1} year^{-1}, far higher than other southern African game reserve. Current ungulate grazer density across the entire park is approximately 87 kg ha^{-1}, with white rhino comprising approximately 40% the total grazer biomass (2000 game count data – Hluhluwe Research Centre).

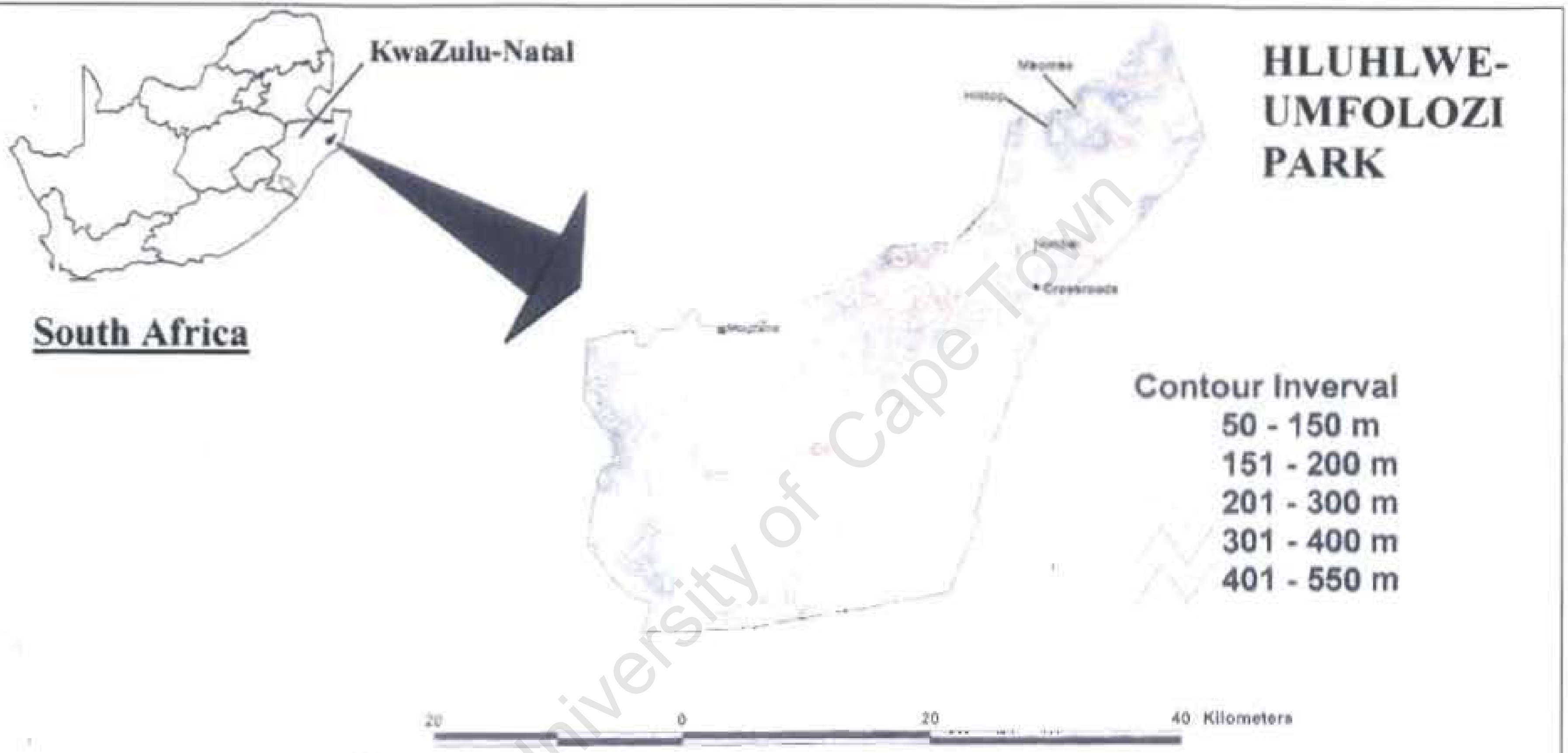


Figure 1.1. Topography of the Hluhluwe-Umfolozi Park. Labelled sites are those used for the mesic-end experiment (Hilltop) and the arid-end experiment (Mbuzane) described in chapter 3, as well as the main sites of the landscape experiment (Mbombe, Nombali, Crossroads) described in chapter 4.

Grazing lawn community characteristics

Species composition

Little phytosociological analysis of the grasses (family Poaceae) of HUP has been done. Much of the pattern regarding grazing lawns is therefore reported from personal observation, or from unpublished data (nomenclature follows Gibbs Russel *et al.* 1990). In areas of intermediate rainfall (approximately 750mm year^{-1}) grazing lawns occur in lightly wooded areas as a mosaic of short, prostrate grass species (in approximate order of abundance: *Digitaria longiflora, Eragrostis superba, Bothriocloa insculpta, Chloris gayana, Dactyloctenium australe, Chloris pycnothrix, Sporobolus nitens, Urochloa mossambicensis, Paspalum scrobiculatum, Panicum coloratum, Cynodon dactylon, Digitaria ternata,*) interspersed with clumps of much taller species (typically *Sporobolus pyramidalis, Themeda triandra* and *Eragrostis curvula*; **Fig. 1.2A**). The former are frequently grazed during the growing season (summer), while the latter are largely ignored, although they often grazed (apparently unintentionally) at the interface between patches. The short grass patches are often interspersed with forbs and, occasionally, *Aristida bipartita*.

In the more arid parts (approximately 650mm year^{-1}), both within the park and in neighbouring communal grazing areas, grazing lawns occur as a more continuous cover of short, prostrate grasses (*Urochloa mossambicensis, Panicum coloratum, Sporobolus nitens, Sporobolus ioclados, Digitaria argyrograpta, Chloris pycnothrix, Eragrostis superba, Chloris virgata, Eragrostis trichophora, Bothriocloa insculpta, Tragus berteronianus, Enneapogon cenchroides, Urochloa trichopus, Eragrostis racemosa*). The species are generally slightly larger, but basal cover lower than on the mesic lawns, and the occurrence of forbs higher (**Fig 1.2B**). Taller, apparently ungrazed species also occur within these communities (*Aristida congesta barbicollis, Perotis patens, Aristida adscensionis*), but their distribution is generally dispersed rather than clumped. In the most arid parts of the park, grazing lawns are apparently excluded by a high intensity of grazing (e.g. around water sources). Here, the herbaceous community is reduced to a sparse cover of the above unpalatable bunch grasses, and forbs.

A third type of grazing lawn occurs under the spreading canopies of larger trees. These occur across the rainfall gradient, but are more common in the mesic parts. Within these permanently shaded communities, the strongly stoloniferous species *Dactyloctenium australe*, can dominate in patches of well over 100m^2, or forms a mosaic with taller grasses, usually *Panicum*

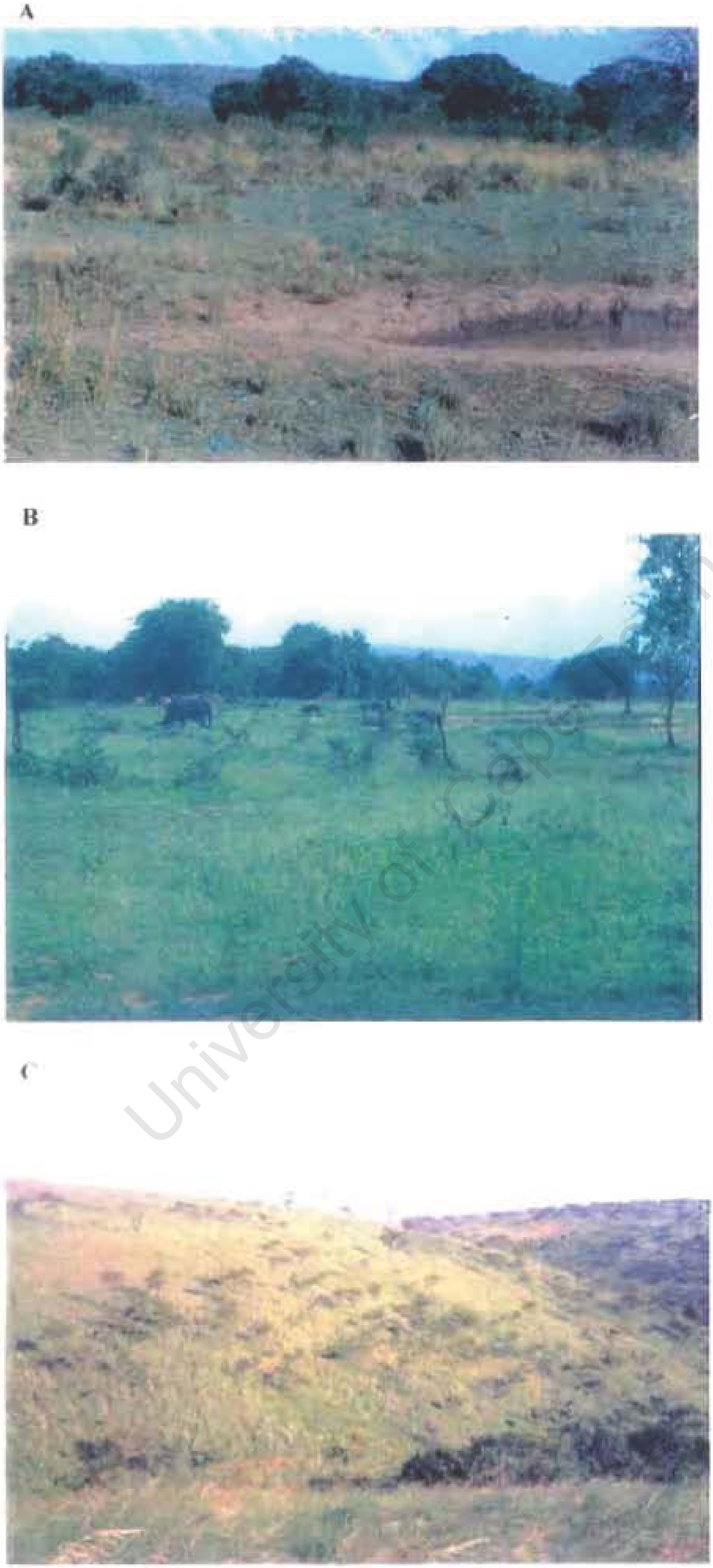


Figure 1.2. Common grass communities in Hluhluwe-Umfolozi Park, South Africa. **A.** A grazing lawn in a more mesic part of the park (rainfall approximately 800 mm year^{-1}). The dominant species on the short patches is *Digitaria longiflora*; patches of bunch grasses are mainly *Sporobolus pyramidalis*. **B.** A grazing lawn in the more arid parts of the park (rainfall less than 700 mm year^{-1}). Dominant species are *Urochloa mossambicensis* and *Sporobolus nitens*. Forbs are common. **C.** Uniform swards of bunch grasses at the upper end of the altitudinal gradient (rainfall approximately 1000mm^{-1}). Dominant species are *Themeda triandra* and *Hyparrhenia filipendula*.

maximum (Swemmer 1998; Roberts 2000). *Chloris gayana*, another stoloniferous species, often occurs on the edges of these shaded lawns. Within all three types of short grasses patches described above, caespitose species which are regularly grazed short can also be found (typically *Themeda triandra* on the unshaded lawns; and *Panicum maximum* on the shaded lawns).

Even in the driest parts of the park, ungrazed (or lightly grazed) communities dominated by tall, caespitose species cover a far greater area than the grazing lawns. The composition of these communities is typical of those found in South African savannas east of the escarpment. In the more mesic areas T*hemeda triandra* and *Hyparrhenia filipendula* are most common (**Fig. 1.1C**); in the drier areas T*hemeda triandra* and *Panicum maximum*. Patches dominated by very tall species (*Hyperthelia dissoluta* and *Hyparrhenia spp.*) sometimes occur, particularly in more mesic areas. These communities are collectively referred to as **bunch grass communities** hereafter.

Herbivore associations

Unpublished dung count data from the Zululand Grasslands Project (ZLGP; www.uct.ac.za/depts/botany/zlgp) confirm observations of the consistent (year after year) presence of grazing mammals on grazing lawns from mid-summer to early winter, and in far greater abundance than in neighbouring bunch grass communities. Species include white rhino (*Ceratotherium simum*), blue wildebeest (*Connochaetes taurinus*), Burchell's zebra (*Equus burchelli*), impala (*Aepyceros melampus melampus*), warthog (*Phacocherus aethiopicus*) and occassionally, buffalo (*Syncerus caffer*). In a study of the Hluhluwe section of the park (the most mesic section) Page and Walker (1978) report that rhino and blue wildebeest were found most frequently in areas of short grass and avoided tall grass swards (dominated by *Themeda triandra*), at the end of the growing season. In a study of the Umfolozi section of the part (generally semi-arid), Downing (1974) found that communities which experienced greatest animal utilization had a high species "constancy" of *Urochloa mossambicensis* and *Panicum coloratum*, and to a lesser extent, *Panicum maximum*, *Sporobolus nitens*, and '*Sporobolus smutsii*' (presumably *Sporobolus ioclados*). The least utilized communities were dominated by tall, caespitose species - *Themeda triandra* particularly, but also *Cymbopogon spp.*, *Panicum deustum*, *Eustachys paspaloides*, *Diheteropogon amplectans* and *Panicum maximum*. Again in the Umfolozi section, Owen-Smith (1988) reports that white rhino concentrated their feeding in the summer in areas of short grass, with favoured species being *Panicum coloratum, Urochloa mossambicensis, Digitaria* spp. and *Sporobolus* spp.

Downing (1974) also described a community which was not heavily grazed, but was considered to be overgrazed due to the frequent occurrence of species considered to be unpalatable (particularly *Bothriocloa insculpta,* but also *Aristida congesta*). Such communities usually have a low basal cover of these grasses but a relatively high cover of forbs (pers. obs.).

Regarding the shaded grazing lawns, it has been observed that white rhino feed regularly in patches of *Dactyloctenium australe* (Owen-Smith 1988; Perrin and Brereton-Stiles 1999). The only other evidence of a concentration of grazers here during the summer, other than personal observation, is that of unpublished dung count data collected in a few of these communities in autumn (Swemmer 1998; Roberts 2000).

Tree associations

Widespread and rapid tree recruitment ("bush encroachment") which has occurred over much of HUP over the past 50 years (Brookes and MacDonald 1983; Skowno *et al.* 1999) does not appear to occur on the grazing lawns. The densities of tall, woody plants on the unshaded lawns is low, and generally consists of short, apparently stunted trees. These appear to act as grazing refugia for the taller, palatable grass species. In the mesic parts at least, there is a turnover of species from lawns to neighbouring bunch grass communities, with less palatable species (such as *Dichrostachys cinerea*, *Acacia nilotica* and *Omorcarpum trichocarpum*) more common on the lawns, and the palatable species *Acacia karoo* less common (Swemmer 1998; Bond *et al.* 2001).

Fire association

Patches of lawn grass do not burn as grazing reduces the standing biomass to little more than stubble by the end of the growing season (pers. obs). In the more mesic parts fires may cross mosaic grazing lawn through the connection of patches of ungrazed bunch grasses.

Grazing lawn distribution

Spatial distribution

Grazing lawns occur in HUP as patches ranging in size from one or two m^2 to many hectares. These patches have been found across the entire altitudinal gradient of the park, but are far more common at lower altitude, and all but absent from the highest altitude grasslands (Swemmer 1998, pers obs., S. Archibald unpublished data; Bond *et al.* 2001). They are not confined to one type of geology, and occur on both dolerite- and shale-derived soils (S. Archibald, unpublished data), and occur on soils varying greatly in texture (Swemmer 1998). In addition, unpublished data collected by the ZLGP indicates little difference in the soil clay content, pH, total nitrogen and total phosphorus between grazing lawns and neighbouring grass communities.

Temporal distribution

All three types of grazing lawn community described above - even the smaller (m^2) patches – persist for at least several years, although there may be considerable accretion or shrinkage at their margins. This is based on personal observation from visits to the reserve over four years, unpublished community composition data of the ZLGP collected over the past three years, and analysis of aerial photography, which indicates that one of the larger lawns in HUP has changed little in extend since 1954. Therefore, while these communities are associated with high concentrations of grazing mammals, there is no evidence that they are transient or "pioneer" communities produced by severe, episodic animal activity (grazing, wallowing or rolling).

Grazing lawn significance

The patterns described above imply four potentially important roles of grazing lawns in the HUP savanna and grassland ecosystems.

1. Secondary productivity – the concentration of grazers in high densities on the lawns, year after year, suggests that the rate of supply of forage to grazers is higher on grazing lawn than other communities in the park.
2. Biodiversity – they contribute significantly to grass species diversity and possibly mammal diversity (Owen-Smith 1989), in that they provide a habitat for specialist short-grass feeders such as warthog and impala which prefer heavily-utilized areas (Wentzel *et al.* 1991). Furthermore, grazing lawns may support unique forb species (R. Uys, unpublished data).

3. Tree distribution – they appear to inhibit the recruitment of common bush encroachment species.
4. Mineral fluxes – as they do not burn, carbon and nitrogen fluxes to the atmosphere may be low, which could prove to be of importance for the sequestration of these minerals in the face of anthropogenic inputs.

The first point is of particular interest, as the management of HUP relies on high rates of secondary productivity to generate income for the park, through the capture and sale of white rhino and other large grazers.

Considering these roles, the question was posed regarding the rather limited distribution of grazing lawns within HUP. If they were more widespread the population growth of economically important grazing species (such as white rhino) might be increased, and the costly problem of bush encroachment reduced. With the advent of global change, potential changes in the distribution of lawns - and the formulation of management strategies to facilitate (or restirct) such changes - could prove vital for achieving the conservation goals of the park. The question: **"what limits the distribution of grazing lawns in HUP?"** was therefore chosen as the basis of the thesis. However, as a large diversity of grazing mammals once roamed many other parts of the world (Owen-Smith 1988), this question was extended to other "grass-grazer ecosystems".

A Broader context – the generality of the grazing lawn pattern

Grazing lawn communities, as described above, have three defining characteristics: dominance by grass species tolerant of frequent grazing (in the growing season), a high rate of forage supply (leading to high secondary productivity), and persistence through time, both in terms of species composition and productivity (implying an equilibrium between grazers and grazing lawns). The final point is particularly important as it distinguishes grazing lawns from transient pioneer communities resulting from the reversal or slowing of grass community succession - a common response to herbivory (Drent and van der Wal 1999). A review of the literature indicated that such communities are not common worldwide and the limited distribution of grazing lawns within HUP seems to be reflected at the global scale. Reviews of studies of grass-grazer systems report that heavy grazing is usually accompanied by 'overgrazing' (Skarpe 1991; Wade and Carvalho 2000), which refers to a state of decreased forage supply and grazer productivity (Wilson and Macleod 1991). Therefore grazing lawns are apparently an unusual alternative state to overgrazing.

Southern Africa

The importance of grazing in determining the species composition of South African grass communities has long been appreciated, although rainfall, soil, fire and tree cover are considered at least as important (O'Connor 1985; O'Connor and Bredenkamp 1997). Almost all local studies interpret community changes in terms of the 'rangelands model': species which become less abundant with grazing (Decreaser species) are considered the most palatable and productive, and species which tend to dominate with heavy grazing (Increaser II and Increaser III species) are considered unpalatable, less productive and indicative of overgrazing (O'Connor 1985; Tainton 1988). In general these studies show that heavy grazing leads to dominance by unpalatable species in mesic areas (e.g. Hatch and Tainton 1990; Morris and Tainton 1992), and dominance by forbs, unpalatable annuals and bare ground in semi-arid areas (e.g. Kelly and Walker 1976; Fuls 1992).

While the occurrence of grazing lawns have never been specifically investigated, potential evidence for their existence can be gleaned from changes in species composition reported in other studies. Certain studies indicate that communities produced by heavy grazing are dominated by short species which are either the same species as grazing lawn grasses in HUP, or have very similar morphology. Furthermore, the species in question are not considered indicative of overgrazing on the basis of palatability but rather low productivity. However, low productivity is usually only assumed, or estimated on the basis of peak standing biomass (a measure of net primary productivity, e.g. O'Connor *et al.* 2001), while it is gross productivity (net production plus growth lost to recurrent grazing) which is the relevant measure (McNaughton 1985a; O'Connor 1985). Therefore some of the 'overgrazed' communities described in the literature may actually offer a high rate of forage supply. Furthermore, forage quality is not often measured (Kelly and Walker 1976) although this could be as important as rates of production. This seems particularly likely in cases where grazers choose to feed on 'overgrazed' patches rather than in neighbouring patches dominated by Decreaser species.

In semi-arid areas of the Highveld potential grazing lawns can be merely transient communities and degenerate to bare soil with sustained heavy grazing, e.g. the community of 'creeping grasses' (*Eragrostis lehmanniana, Digitaria argyrograpta* and *Cynodon dactylon*) described by Fuls (1992). Alternatively, they may show particularly low productivity (even when defoliaiton losses are included in the measure of productivity), such as the 'poor condition' community described by Snyman (1999), which was dominated by *Cynodon hirsitus* and *Tragus koelerioides*. In both cases the poor growth of the potential grazing lawns is associated with a low basal cover, reduced infiltration, greater soil evaporation, greater run-off and increased soil erosion (Fuls 1992; Snyman 1998).

In some studies however, it seems that potential grazing lawns evident in community composition descriptions may posses all of the grazing lawn characteristics mentioned above:

- Gillard (1965) was perhaps the first to challenge conventional overgrazing ideas and argued that since heavy grazing led to the same floristic changes as fertilization, 'primary' South African highveld grass communities were more productive than 'climax' ones (cited by O'Connor and Bredenkamp 1997). Working in the same Highveld grassland, Jones (1964) reported that these primary communities, which occur on heavily grazed old-fields, were dominated by *Cynodon dactylon* and *Eragrostis curvula*, the former being a short, stoloniferous species of remarkably similar morphology to *Digitaria longiflora* (a species common on the grazing lawns in the more mesic parts of HUP).
- Again on the highveld, a model of 'degradation' by Bosch and Theunissen (1992) indicates that *C. dactylon* dominates communities with 'severe overgrazing', especially in the wetter parts (> 750 mm year^{-1}). While this species is considered palatable, these heavily-grazed communities are considered degraded of low forage supply. However, a pasture experiment conducted in a mesic part of the highveld has shown that *C. dactylon* can produce over 5000 kg ha^{-1} (gross above-ground productivity) even when regularly grazed (I'Ons 1974).
- Parsons *et al.* (1997) report that the stocking rate of communal grazing lands in mesic part of Mpumalanga was more than double that in neighbouring commercial cattle farms and private game farms, and had been for many years. The communal areas, dominated by short, stoloniferous species (*C. dactylon* and *Digitaria eriantha*), had a

higher basal cover, an equal abundance of perennial species and showed no signs of soil erosion.

- deBruyn (1998), in a comparison of communal and commercial rangelands in the grasslands of the Eastern Cape, found that although the communal rangelands had been 'overstocked' for close to 150 years, they had higher basal cover, high leaf to stem ratios, and similar gross above-ground production compared to neighbouring commercial farms. Species dominant in the communal grazing areas were again *C. dactylon* and *D. eriantha.*
- Novellie (1990), working in the grasslands of the Mountain Zebra National Park, Eastern Cape, found that certain grazers (black wildebeest and springbuck) consistently concentrated their feeding on patches on *Cynodon incompletus,* a short stoloniferous and rhizomatous species, which led him to describe these communities as grazing lawns.
- Novellie (1988) and Novellie *et al.*'s (1991) investigations of grass communities of the Addo Elephant National Park, Eastern Cape, found that frequent grazing over many years led to the replacement of bunch grass communities (dominated by *Themeda triandra* and *Cymbopogon spp.*) with communities dominated by short species, some stoloniferous (*D. eriantha, Panicum stapfianum* and *Eragrostis obtusa*).
- In a semi-arid savanna in the nothern Province, the Increaser II communities which dominated heavly-grazed treatments in the Towoomba grazing trial analysed by Smit and Rethman (1992) consisted of short palatable species including *Urochloa mossambicensis, D. eriantha, Michrocloa caffra, Chloris virgata* and *Sporobolus nitens*. While the net primary productivity of these communities, in the absence of grazing, was lower than lightly grazed communities dominated by Decreaser species, their gross productivity under conditions of frequent grazing was no measured.
- A number of studies which describe the pattern of 'patch selective' grazing in the mesic grasslands of KwaZulu-Natal report that grazers prefer 'overgrazed' patches dominated by short (but frequently grazed) species such as *Michrocloa caffra* (Hatch 1991; Lutge 1995; Lutge *et al.* 1996).
- Finally, in a study in which some measure of forage supply was made (animal gains per year), Fynn and O'Connor's (2000) analysis of a ten year grazing trial in northern KwaZulu-Natal showed that cattle gains (per hectare) were not affected by stocking rates The more heavily-grazed treatments showed a change in grass species composition

with short, prostrate species such as *Urochloa mossambicensis, Sporobolus nitens, Digitaria argyrograpta* and *Sporobolus ioclados* often dominating. Fynn and O'Connor do point out, however, that in years of drought animals gains fell more for these communities than the lightly-grazed ones.

Africa

The very concept and original definition of grazing lawns stems from studies of the Serengeti ecosystem in east Africa (McNaughton 1979, 1983, 1985a). McNaughton described the occurrence of a number of short-grass communities, and demonstrated that concentrated grazing by the very high density of grazers in the Serengeti leads to the formation of these communities, and that they are more productive than ungrazed communities. There are many similarities between descriptions of the grazing lawn distributions in the Serengeti (McNaughton 1979; Belsky 1983; McNaughton 1983) and patterns in HUP. In both systems, grazing lawns:

- occur across a similar altitudinal gradient and a similar rainfall gradient;
- are more common at the arid end of this gradient (but dominate completely in areas under 700mm in the Serengeti, which never occurs in HUP);
- occur as a patch mosaic together with "mid grasses" (bunch grasses), or are replaced by bunch grass communities completely, in more mesic areas (> 825 mm rainfall a year in the Serengeti).

The only other example found of grazing lawns in Africa comes from the results of grazing trial in neighbouring Uganda (Harrington and Pratchett 1974a, b). Harrington and Pratchett report that the highest stocking rate led to a replacement of a community dominated by *Themeda triandra* and *Hyparrhenia filipendula* with grazing lawns dominated by *Brachiaria decumbens*, and produced the greatest animal gains per hectare. The lack of further examples of African grazing lawns may be due to a lack of both ecological research and the disappearance of grazing lawns following the decimation of indigenous grazer populations in most parts of the continent. For example, Smart *et al.* (1985) report the disappearance of *Chloris gayana* and *Sporobolus pyramidalis* (species characteristic of some grazing lawns in HUP) from two sites in the Murchison Falls National Park, Uganda following widespread poaching of large herbivore during a civil war (only very tall grasses such as *Hyparrhenia rufa* increased in abundance during this period). In addition, the tendency to measure only net primary productivity may have concealed the presence of grazing lawns in certain African studies (e.g. Edroma 1984), as was argued for South Africa.

World-wide

No clear descriptions of grazing lawns were found in the international rangelands literature. In a meta-analysis involving over 100 grass communities from around the world, Milchunas and Lauenroth (1993) found that above-ground net primary productivity (ANPP) was almost always lower for grazed communities, compared to ungrazed ones, and that this difference increased with the estimated loss of ANPP to grazers. Again however, a lack of gross productivity and / or secondary productivity measurements raises the possibility that grazing lawns were overlooked.

Reports of grazing lawns were not found for the few protected areas where naturally occurring grazers have survived either. While some examples of potential grazing lawns consisting of introduced grass species were found, these have not been included here. Community changes in response to grazing by re-established herds of bison in tallgrass prairie, North America, do involve a certain degree of species turnover to more grazing-tolerant species (Hartnett *et al.* 1996). However, such communities appear to be transient (as they are not re-grazed consistently for many years) and do not offer consistently higher productivity (net productivity appears to decline after a few years - Knapp *et al.* 1999). The lack of grazing lawns beyond Africa may be a result of the extinction of many large herbivores from North America and Eurasia at the end of the Pleistocene, and earlier in South America and Australia (Owen-Smith 1989). In support of this argument, a simulation model by Zimov *et al.* (1995) suggests that the currently moss-dominated tundra of Beringia was a heavily-grazed, grass-dominated system prior to the Pleistocene extinctions. Mack and Thompson (1982) argue that steppes west of the Rocky mountains in North America are dominated by caespitose grasses because of a lack of large grazing herbivores through the Holocene.

From the above it is clear that grazing lawns are unusual, and that next to the Serengeti, HUP may contain the best examples of such communities. Studying the limited distribution of lawns in HUP provides an opportunity to identify general ecological conditions necessary for the formation and persistence of grazing lawn communities, and to determine whether their absence is a result of constraints imposed by the (abiotic) environment, or simply a lack of grazing (which can often be attributed to anthropogenic reductions of grazer densities).

CONCEPTUAL FRAMEWORK

Philosophical approach

To determine which processes control the distribution of a community, one is faced with a choice of two broad philosophical approaches, holism or reductionism. A holistic approach would involve finding correlations between the distribution of grazing lawns and environmental variables considered important. From the outset, this approach was decided against, for the two reasons.

1. The relationship between the community patterns described above and environmental variables is not well understood, as little plant ecological research has been done in HUP. Only coarse-scale measurements of rainfall, geology and grazer numbers were available at the outset of this study, and attempting to gather more environmental data was considered beyond the scope of the topic chosen for the thesis. In addition, the distribution of the grazing lawns has not been quantified. Many of the grazing lawns occurs as patches of only a few m^2, and their distribution could not be detected from aerial photographs (although some success has since been had using LANDSAT satellite imagery – S. Archibald; unpublished data).
2. Once the important environmental variables are identified, predictions can only be made for the range over which these variables were sampled. Extrapolation beyond this range makes assumptions regarding the linearity of their effects, which could lead to large errors.

A reductionist approach

Adopting a reductionist approach is potentially more complicated, as one encounters a large increase in complexity when moving one down one (or more) mechanistic levels. With reference to the distribution of grazing lawn communities, reducing grazing lawn communities to their component parts (grazing lawn species) exposes a wealth of new complexity in the form of:

1. The number of species involved.
2. The number of resources (light, water, CO_2 and 14 minerals) and stresses (defoliation, physical damages, and extreme temperatures, pH, and humidity) affecting the growth of each species.
3. The interactive effects of these resources and stresses (i.e. the effects of one can be contingent upon the supply rate of others)
4. Spatio-temporal variation in the supply of these resources and stresses, produced by abiotic factors and the growth of other species (which emerges as competition, or facilitation).

Despite this complexity, a reductionist approach was adopted and maintained throughout the study, on the basis that previous ecological research (in grass-grazer systems and others) suggests that much of this complexity is redundant, i.e. only certain ecological processes controlling plant distributions.

Coping with complexity

If much of the above complexity is redundant, the task of understanding the distribution of grazing lawns is greatly simplified, provided one can determine which of the plethora of ecological processes involved are the important ones. The adoption of a reductionist approach, in this thesis, therefore incorporated the assumption that certain ecological processes are of disproportionate importance in controlling the distribution of the grazing lawns, i.e. the rate of supply of only certain resources or stresses is responsible for the absence of grazing lawns from certain environments. If the rate of supply of different resources / stresses correlate strongly, they can be treated as a single factor, bringing further simplicity. The term **key processes** is used to refer to supply of those resources / stresses (or groups of correlated resources / stresses) which are considered to be of fundamental importance. This 'key processes' approach also assumes that all the species which comprise the community under investigation (in this case, lawn grasses) are affected by supply of the key resources / stresses in the same way.

Finding 'key processes'

Applying the concept of key processes requires three steps:

1. a hypothesis of which resources / stresses are key,
2. determining the rate of supply of the relevant resource / stress in the system(s) of interest,
3. determining the effect of that rate of supply on the growth of the relevant species.

The first point is discussed below. The second point (measuring the availability of particular resources / stresses on grazing lawns in HUP) has not been pursued further. Rather, rates have been estimated on the basis of observation, or inferred from studies of other grass-grazer systems. The third point (measuring the growth responses of the HUP grazing lawn grasses to the proposed key processes) forms the bulk of the thesis and is dealt with in subsequent chapters.

Key processes for grazing lawns

A conceptual model of a general 'grazing lawn' system was developed to illustrate which ecological processes, out of the many present in a grass-grazer system, are important in controlling the distribution of grazing lawns (**Fig. 1.3**). Three ecological processes are considered necessary to switch a grass-grazer system to a stable grazing lawn state: grazing during the growing season which is both frequent and non-selective, and a greater tolerance of this grazing by lawn species than other grasses (bunch grasses). A high frequency of grazing involves positive feedback and implies an attraction of grazers to the lawn species. The model therefore represents an example of 'self-organization', the concept in which the processes which form a pattern are themselves reinforced by that pattern, and one which is increasingly being used to model community distributions for a range of different ecosystems (Holling *et al.* 1996; Rietkerk *et al.* 2002). Finally, the model implies that in the absence of grazing, lawn grasses are excluded from the system because they cannot compete with the bunch grasses.

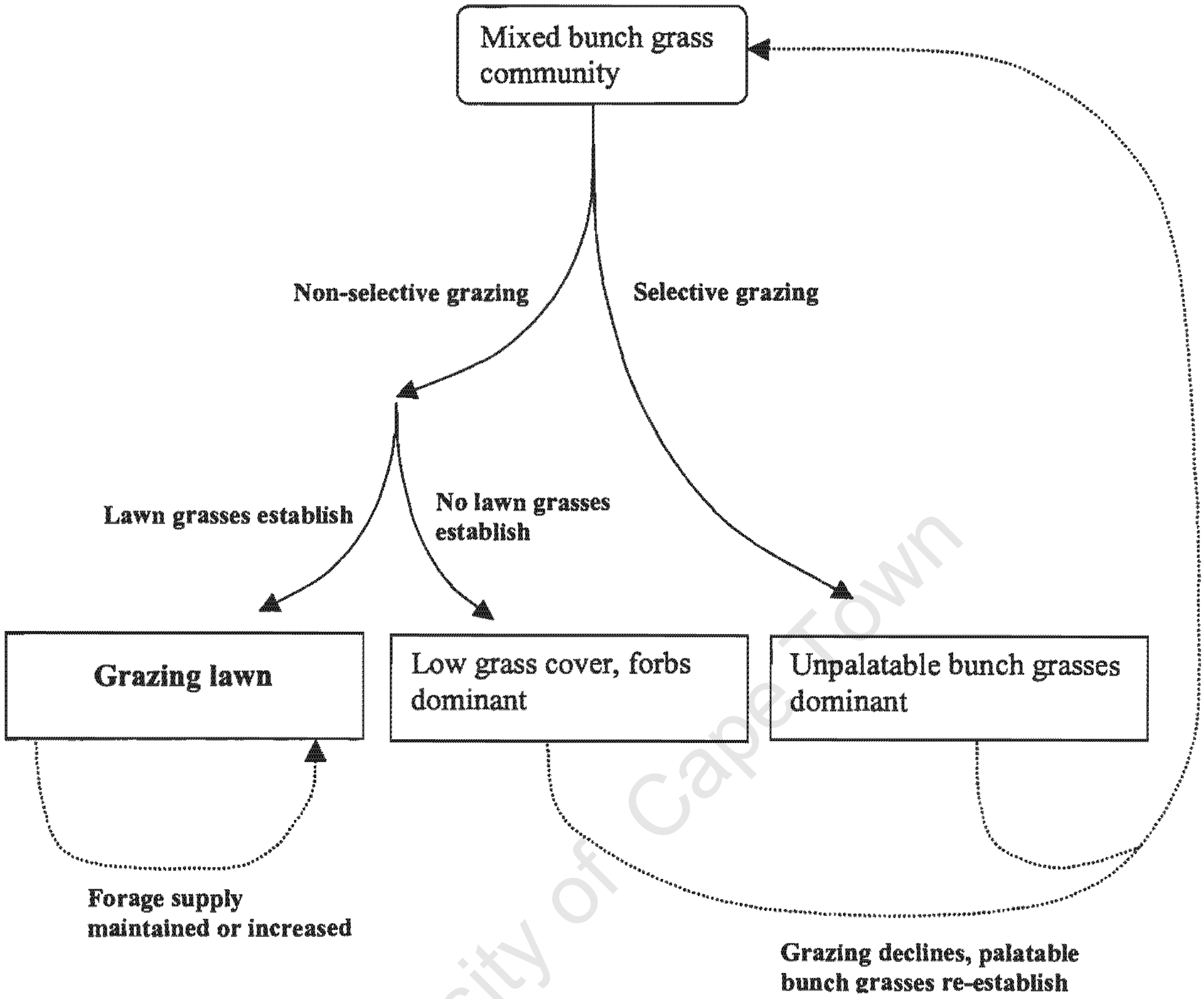


Figure 1.3. A conceptual model of how grazing can drive a grass-grazer system to different states, including a stable grazing lawn state. Non-selective grazing together with the establishment of grazing-tolerant lawn grass species leads to the formation of a grazing lawn. Superior forage supply (or other factors such as reduced risk of predation) on the grazing lawns results in a positive feedback loop between lawn grass establishment and grazing, and the maintenance of the grazing lawn state. Non-selective grazing without the establishment of lawn grasses results in elimination of all grasses, resulting in bare ground and / or dominance by forbs. This leads to a decline in grazing and a return to a bunch grass community (provided that bunch grasses can re-establish). Selective grazing eliminates only palatable grasses, resulting in dominance by unpalatable bunch grass species. This also leads to a decline in grazing, and a return to a mixed bunch grass community (provided that palatable bunch grasses can re-establish).

A definition of grazing

For the purpose of this model (and the remainder of the thesis) **grazing** is considered to include the effects of both defoliation and trampling. Also, the term is used to refer to the loss of *live* material only - loss of dead material (standing dead) which occurs with grazing in the non-growing (dry) season is not involved. Furthermore, grazing tolerance should not be confused with grazing avoidance. The latter refers to the deterrence of grazing through unpalatability, or growing beyond the reach of grazers. Although grazing lawn grasses actually show a combination of both traits, in that a significant portion of stems and leaves are produced so close to the ground that they are out of the reach of grazers, they still yield some forage to grazers (pers. obs.).

'Lawn grasses' and 'bunch grasses' as functional groups

In the above model lawn grasses represent different functional groups (*sensu* Gitay and Noble 1997). The grazing lawn species are assumed to share similar 'functions'(a high tolerance of grazing and an ability to attract grazers), while bunch grasses share others (a low tolerance of grazing and an ability to outcompete lawn grasses in the absence of grazing). This may not be true. For example, unpalatable species found on grazing lawns would not share the above functions but have others (e.g. grazing avoidance) which enable them to survive on grazing lawns. In this case, the lawn grass category would need to be split into different functional groups. Similarly, the subdivision of the bunch grass category into palatable and unpalatable species was necessary to explain the formation of grazer-induced states other than the 'grazing lawn state' (**Fig. 1.3**). As the remainder of the thesis deals with the 'ecological functioning' of lawn grasses only, subdivision of the lawn and bunch grasses categories was not considered necessary.

Model validity

Most conceptual or analytical models which deal with grazing involve only negative feedback, with grazing leading to reduced grass productivity and ultimately a reduction in further grazing (e.g. van de Koppel *et al.* 2002). However, the idea that grazing leads to more grazing is not a new one (McNaughton 1979; Owen-Smith 1988) and has some empirical support e.g.(McNaughton 1985a). From a plant perspective, the idea that certain plant species rely on grazing to eliminate superior competitors is not new either: Goldberg (1997) describes the process generally as 'apparent competition'. It has been applied to grass-grazer systems specifically by McNaughton (1979), who used the term 'obligate grazophils' to refer to lawn grasses which were observed to disappear with the exclusion of grazers. Previous application of such ideas does not necessarily mean the model presented above is appropriate for the situation in HUP. Therefore, before expanding the model to incorporate further key processes, the likelihood of the above key processes occurring in HUP is considered.

1) The grazing tolerance of lawn and bunch grasses

Long-term grazer exclusion experiments in the Serengeti provide clear evidence of the lower tolerance of bunch grasses to grazing, with community composition switching from lawn grass species to bunch grass species following the exclusion of grazers (Belsky 1983; McNaughton 1985a). This process has also been suggested for HUP, based on phytosociological work in the Umfolozi section of HUP (Owen-Smith 1988 cites Downing 1979). Recently, ten grazing exclosures were erected across the whole park (ZLGP; www.uct.ac.za/depts/botany/zlgp) and results so far provide good evidence for this process. Three years since the exclusion of large grazing mammals, the peak standing biomass and aerial cover of bunch grasses has increased dramatically, while lawn grasses have declined (**Fig. 1.4A**). Species composition data (in absolute terms) is being collected annually and formal analysis of this will almost certainly show the statistical significance of the observed changes.

The problem of contingency

Bunch grasses may well be excluded by grazing in certain situations but the response of a plant to defoliation is contingent on a range of environmental factors (summarized by McNaughton 1979). Both pot and field experiments have shown that grass growth responses to defoliation are affected the height and frequency of clipping (Pretorius *et al.* 1974; McNaughton 1979; McNaughton *et al.* 1983; Edroma 1985), the supply of nitrogen (discussed in chapter 2), and the supply of water (McNaughton *et al.* 1983; Coughenour *et al.* 1985a; Ganskopp 1998). In some cases, defoliation can even act as a resource, rather than a stress, with clipping leading to increases in growth (McNaughton 1979; McNaughton *et al.* 1983; Edroma 1985; Maschinski and Whitham 1989). Janse van Rensburg and Bosch (1990) show how these effects translate into differences at the population level, with slight differences in soil factors (such as "% stoniness") altering the effect of grazing on the relative abundance of a range of grass species over long periods. A similar example comes from the responses of *Digitaria eriantha* to grazer exclusion. This species has been shown to increase with heavy-grazing in the Eastern Cape and Northern Provinve (Novellie 1988; Smit and Rethman 1992; O'Connor 1994), but in HUP it has increased inside grazing exclosures (ZLGP, unpublished data). Contradictory patterns such

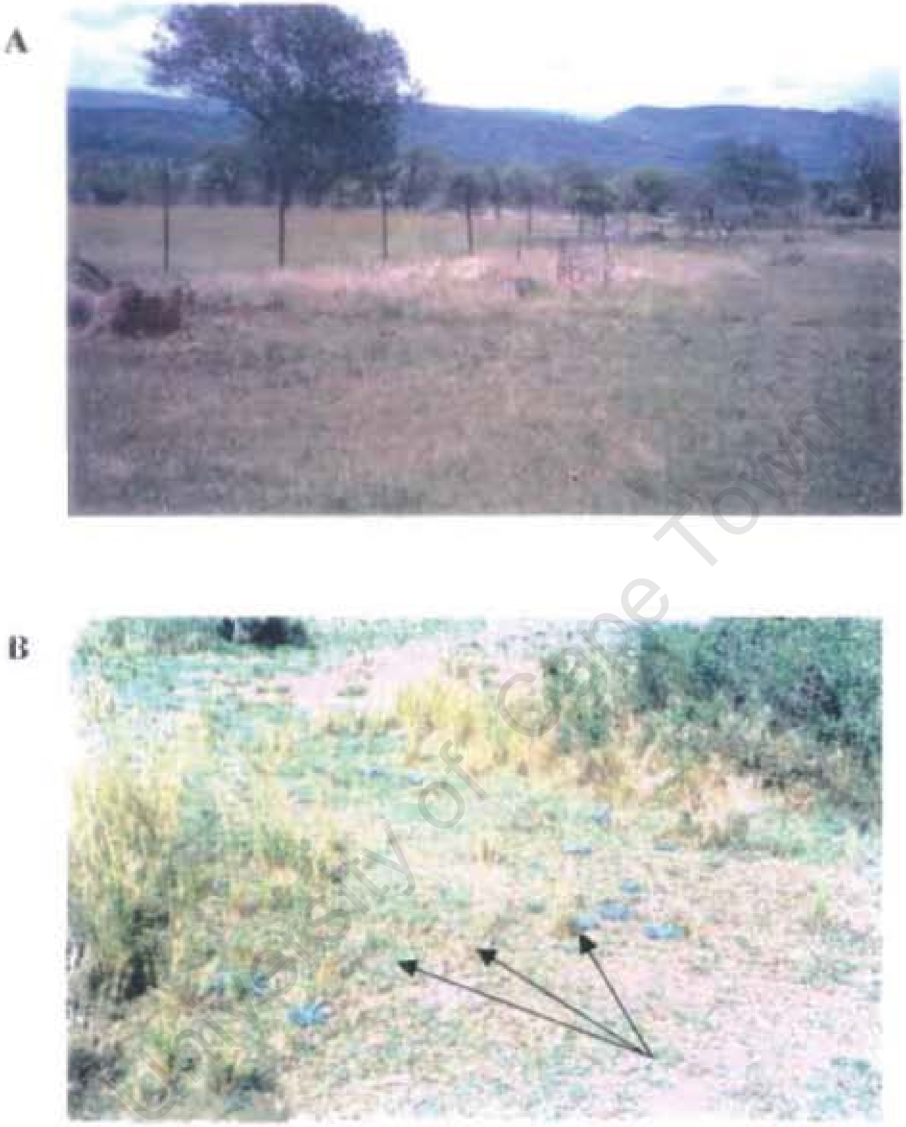


Figure 1.4. A. The effects of the exclusion of grazers in Hluhluwe-Umfolozi Park, South Africa Approximately two years after the erection of this grazing exclosure (at the Thoboti site of the Zululand Grasslands Project), bunch grasses dominate what was once a grazing lawn community The dominant species within the exclosure is *Themeda triandra*. Outside, frequent grazing maintains patches of lawn grasses, mainly *Digitario argyrograpta* and *Panicum coloratum*. **B.** A patch of *Digitaria longiflora* dominated grazing lawn in a more mesic part of Hluhluwe-Umfolozi ('Crossroads' area). Bunch grasses surrounding the lawn are mostly *Sporobolus pyramidalis* and are lightly grazed. Arrows show isolated tufts of *S. pyramidalis* within the lawn patch that have been uniformly grazed to a short height.

as these have led to criticism of the use of the "Decreaser" and "Increaser" classifications (Mentis 1983; Janse van Rensburg and Bosch 1990).

Despite these examples it is maintained that grazing, and not some other process, causes the replacement of bunch grasses by lawn grasses in HUP. Firstly, the ten grazing exclosures in HUP, where this process has been observed, cover a range of environmental conditions. Secondly, many of the common bunch species in HUP have been reported to suffer reduced abundance, in repsonse to heavy grazing, in other areas: *Themeda triandra* (Mentis 1983; O'Connor 1985; Novellie 1988; Danckwerts and Nel 1989; Hatch and Tainton 1990; Novellie 1990; Fuls 1992; Morris and Tainton 1992; O'Connor and Pickett 1992; Smit and Rethman 1992; O'Connor 1994), *Heteropogon contortus* (O'Connor 1985; O'Connor and Pickett 1992; O'Connor 1994), *Setaria sphacelata* (Fuls 1992) *Cymbopogon plurinodis* (O'Connor 1985; Novellie 1988; Novellie 1990), *Hyparrhenia filipendula* (O'Connor 1985), *Eustachys paspaloides* (O'Connor 1985) and *Eragrostis curvula* (Fuls 1992). Regarding lawn grasses the following species, common in HUP, are reported to dominate heavily grazed communities (or increase with increasing grazer density) in other areas of southern Africa: *Urochloa mossambicensis* (O'Connor 1985; O'Connor and Pickett 1992; Fynn and O'Connor 2000), *Sporobolus nitens* (O'Connor and Pickett 1992; Fynn and O'Connor 2000), *Chloris virgata* (O'Connor and Pickett 1992; Fynn and O'Connor 2000), *Digitaria argyrograpta* (O'Connor 1985; Fynn and O'Connor 2000), *Sporobolus ioclados* (Fynn and O'Connor 2000), *Dactyloctenium australe* (Fynn and O'Connor 2000), and *Bothriocloa insculpta* (O'Connor 1985).

2) Selective versus non-selective grazing

Even if the bunch grasses cannot tolerate grazing, grazing lawns would not form unless the majority of grasses in the system experience grazing durng the growing season. Only personal observation can be offered as evidence for this. This issue certainly deserves more attention, however, as non-selective grazing is often accepted as the mechanism underlying changes in community composition in response to grazing. Anderson and Briske (1995) provide a good example of the non-selective grazing mechanism – in a field experiment in a mesic prairie (North America) selective clipping of the palatable species *Schizachyrium scoparium* resulted in its replacement by co-occurring unpalatable species. However, with non-selective clipping, this species maintained its dominance. (Flemmer *et al.* 1999) provides a similar example.

In HUP selective grazing certainly does occur, resulting in patches of unpalatable bunch grasses, particularly *Eragrostis curvula* and *Sporobolus pyramidalis*, on grazing lawns in the more mesic parts. These species have been shown to increase with grazing in other studies, and it is maintained that this is a result of selective grazing (Mentis 1983; Morris and Tainton 1992). Barnes *et al.* (1984) categorized these two species as "moderately unpalatable" to cattle on the basis of measures of animal intake, while O'Reagain (1993) found that they suffered far fewer bites from sheep, as compared to a range of other local species (including the grazing-tolerant species *C. dactylon*). However, non-selective grazing also occurs, in the matrix of lawn grasses which surrounds the patches of tall unpalatable species, and many individual tufts (and presumably seedlings) of these species have been observed to suffer severe grazing here (**Fig. 1.4B**). The bifurcation between a grazing lawn state and a unpalatable-dominated state in response to intense grazing (**Fig. 1.3**) therefore appears to develop at a fine resolution (a few square metres) in the more mesic parts of HUP. It is likely that the wide-mouthed grazer species, such as white rhino, blue wildebeest and warthog, are unable to feed selectively and are responsible for the non-selective grazing. For example, white rhino have been observed to eat *Bothriocloa insculpta* (Owen-Smith 1988), another species avoided by cattle (O'Connor 1992). Furthermore, while O'Connor found *Bothriocloa insculpta* to be less frequently grazed than co-occurring bunch grass species such as *Themeda triandra*, *Setaria incrassata* and *Heteropogon contortus*, tuft structure and previous grazing history had a greater influence on grazer selection by cattle than species. This suggests that if an "unpalatable" bunch grass is grazed before it reaches a certain size (as may happen accidentally with a high density of wide mouthed grazers) it is likely to be acceptable to all grazers thereafter.

3) The attraction of lawn grasses to grazers

Grazers could choose to concentrate their summer feeding on grazing lawns for two reasons: to reduce the risk of predation or because of a superior supply of forage (McNaughton 1984). Evidence for the former process is lacking and seems unlikely for grazers such as the white rhino. The latter process has been investigated quite thoroughly following suggests by McNaughton (1979) that grazers can optimize their food supply through grazing. In the Serengeti, grazing lawn forage has been shown to be superior in terms of rate of supply(i.e. gross productivity - McNaughton 1984, 1985b) and in terms of the concentration of many minerals important to grazers (McNaughton 1990). Even if quantity of forage supply is no greater on grazing lawns, grazers could still obtain superior forage from lawns because of structural differences, i.e. the ratio of live:dead and of leaf:structural material (**Fig. 1.5**). A simulation model of daily mass gains by grazers based on the live:dead and stem:leaf ratios of forage grasses by Owen-Smith (2002) found that grazers achieved the greatest gains (in the mid and late summer) from grazing lawn grasses.

Gross primary productivity data currently being collected using monthly, mobile grazing exclosures on ten grazing lawns in HUP (ZLGP) should soon reveal whether forage on these lawns is superior to that from surrounding bunch grass communities, in terms of quantity, mineral concentrations and live:dead and leaf:structural ratios.

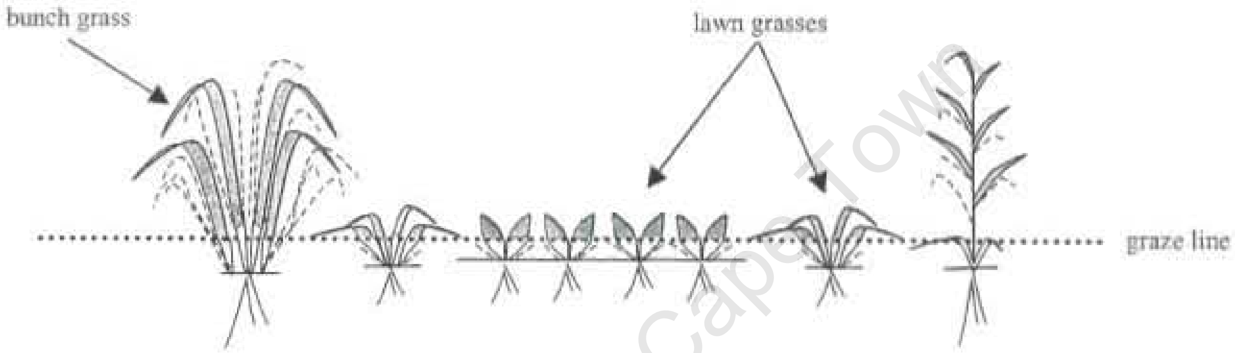


Figure 1.5. Diagrammatic representation showing why lawn grasses may be more tolerant of grazing, and why grazers may obtain superior forage from grazing lawns. Provided grazers are not selective and graze all grasses down to a similar height, the taller bunch grass species suffer more with each grazing event as a result of having proportionately more leaf area above the height to which grazers feed (the graze line). The shorter, prostrate lawn grass species are able to maintain a large leaf area below the graze line (without incurring self-shading). Forage supply is superior from the lawn grasses, as a result of a smaller proportion of culms (|) and dead leaves (\ ) consumed.

4) The exclusion of lawn grasses by bunch grasses

If lawn grasses were able to co-exist with ungrazed bunch grasses, then the elimination of bunch grasses by grazing would be of little significance to the establishment of grazing lawn communities. The disappearance of lawn species after the erection of grazing exclosures in HUP provides good evidence lawn grasses are in fact outcompeted, with light being the obvious resource for which competition occurs (e.g. an ad-hoc measurement of the transmission of photosynthetically active radiation through a fully developed canopy of *Themeda triandra* bunch grass produced a mean transmission of only 7.4 %, SD: 2.3%).

More key processes

While regular non-selective grazing may well explain the occurrence of grazing lawns, it does not explain their absence from so much of HUP (and many other grass-grazer systems) where grazers such as white rhino, blue wildebeest and warthog are free to roam. Even in the most mesic parts of HUP (receiving approximately 1000mm rainfall per year), grazing can maintain patches of bunch grasses at a height of less than 5cm for an entire growing season (pers. obs.). It appears that even with a potentially unlimited frequency of non-selective grazing there are certain environmental conditions that will not allow for the formation and / or persistence of grazing lawns. In other words, it is suggested here that there are additional key processes, over and above frequent, non-selective grazing, which control the distribution of grazing lawns. Determining what these key processes are is the central aim of this thesis, and constitutes the bulk of the following chapters.

To answer this question, two approaches have been used. Firstly, investigations were made regarding which resources / stresses are supplied at relatively high rates on existing grazing lawns. Research in other grass-grazer systems suggests that the supply of minerals, from soil to plant, is accelerated by heavy grazing (particularly nitrogen – chapter 2). Secondly, on the assumption that lawn grasses are optimized (in the evolutionary sense), investigation were made regarding any trade-off's associated with the distinguishing grazing lawn 'functions' (grazing-tolerance and the ability to supply superior forage). Such trade-offs result from the functional cost of acquiring a certain trait and there are many evolutionary ecology studies which provide examples (Begon *et al.* 1990). If lawn grasses do posses 'trade-off' traits, the one can speculate that these underlie key processes which restrict the distribution of the functional group.

Hypotheses

The most likely additional key process controlling grazing lawn distribution is hypothesized to be a high supply of nitrogen (from soil to plant). This is based on research of McNaughton that grazing accelerates nutrient cycling (McNaughton *et al.* 1997a) and speculation that a trade-off associated with grazing tolerance is a high demand for nitrogen. This idea is discussed and tested in chapter 2. Chapter 3 investigates the hypothesis of nitrogen limitation further, and explores another hypothesis, namely that a low supply of water is a key process which restricts the distribution of lawn grasses in semi-arid areas. This is based on firstly, the literature cited above which indicates that heavy grazing leads to a low basal cover of all grasses, and dominance by forbs, in many semi-arid areas of southern Africa. Secondly, the absence of grazing lawns from the most arid parts of HUP. Chapter 4 considers the lack of the evidence obtained in chapters 2 and 3, regarding the importance of a high N supply for lawn grasses in the most mesic parts of HUP, and tests the importance of soil resources in general.

CHAPTER 2

NITROGEN AND LAWN GRASS GROWTH

This chapter investigates the response of lawn grass growth to nitrogen (N). The overarching hypothesis tested is that lawn grasses show a similar, large response to N, relative to other grass species. As this hypothesis incorporates a great deal of a complexity, the chapter has been divided into three sections. Section 1 discusses evidence for the hypothesis that lawn grasses are adapted to grow under conditions of high N supply, and explores the complexity involved in measuring a plant's response to N supply. Section 2 gives the results of experiments which test the effect of the chemical source of mineral N on lawn grass growth. Finally, section 3 gives the results of experiments which test the effect of the rate of supply of N on lawn grass growth.

SECTION 1

THE POTENTIAL IMPORTANCE OF NITROGEN

N supply and demand

The literature provides evidence that firstly, the rate of supply of N from soil to plant increases with grazing intensity and secondly, grazing-tolerant grasses have a high demand for N.

Grazing and N supply

The supply of inorganic (or mineral) N, measured as net mineralization with the use of *in situ* cores, has been shown to increase with grazing in the grass-dominated ecosystems of the Serengeti (McNaughton *et al.* 1997a), Yellowstone National Park in the U.S.A. (Frank and Groffman 1998b; Tracy and Frank 1998; Frank *et al.* 2000) and tallgrass prairie in the U.S.A. (Knapp *et al.* 1999; Johnson and Matchett 2001).There are a number of ways in which grazing may alter soil processes and increase the supply of inorganic nitrogen.

Litter inputs

It has been suggested that grazing decreases the ratio of carbon to nitrogen (C:N) in plant litter, which leads to higher rates of net N mineralization (Holland and Detling 1990; Tracy and Frank 1998). A simulation model by Holland *et al.* (1992) provides evidence for such a mechanism. Using the CENTURY nitrogen cycling model together with data from a prairie grassland grazed by prairie-dogs, Holland *et al.* show an increase in inorganic N supply as a result of grass regrowth after grazing having a lower C:N ratio, leading to a lower C:N ratio of soil organic matter and higher rates of net N mineralization. Furthermore, the study by Johnson and Matchett (2001) found that increases in net N mineralization that resulted from grazing were accompanied by a decrease in the C:N ratio of grass root tissue.

N in animal wastes

With grazing, a large amount of N in the above-ground (AG) parts of grasses is returned to the soil as animal waste (mainly dung and urine) which is a more labile form of organic N than plant litter, and therefore more readily mineralized (Jarvis 2000). Day and Deitling (1990) found that both AG and below-ground (BG) production (and regrowth) of a rhizomatous grazing-tolerant species was enhanced by natural bison urine patches (although it was not for a co-occurring bunchgrass species). In addition, analysis of the relative abundance of the ^{15}N stable isotope in grass leaves suggests that much of the N that grasses in heavily grazed systems utilize is from dung (Kerley and Jarvis 1996). Finally, it has been suggested that plants can access N directly from urine, and bypass the microbial decomposition part of the N cycle (McNaughton *et al.* 1997b). This is based on evidence for the conversion of urea to ammonia in the absence of microbial activity (implying that presence of free urease) in soils collected from the Serengeti.

Rhizodeposition

If grazing results in an increase in rhizodeposition (the exudation, secretion and sloughing of soluble organic compounds from root to rhizosphere) then it may accelerate the release of inorganic N from soil microbes long before changes in plant litter and animal wastes do (Tracy and Frank 1998; Dawson *et al.* 2000; Hamilton III and Frank 2001). Rhizodeposition has been shown to increase after defoliation for a number of pasture species (Dawson *et al.* 2000). Furthermore, with an isotope tracer experiment using ^{13}C labelled CO_2, Hamilton III and Frank (2001) were able to show a direct link between clipping, increased rhizodeposition and the mineralization of organic N. In the space of a week, defoliation of the grazing-tolerant grass *Poa pratensis* resulted in a large increase in not only the release of organic compounds into the rhizosphere, but also rhizosphere microbial C mass, rates of net N mineralization, and plant uptake of N.

Dawson *et al.* (2000) provides three plausible explanations for why clipping would stimulate rhizodeposition:

1. After defoliation, degradation of C and N storage compounds in roots during remobilisation may transiently increase the concentrations of sugars and amino acids, promoting a diffuse release of the exudates.
2. Defoliation reduces the energy status of roots, which perturbs the reuptake of root-released organic compounds.

3. Physical damage caused by defoliation may initiate an electrical action or slow wave potential propagated through the plant, which is capable of depolarising root transmembrane potentials and interfering with reuptake of rhizodeposits.

Hamilton III and Frank (2001) suggest that the reason rhizodeposition increases mineralization is that a release of inorganic N follows the spurt of microbial activity that accompanies increases in rhizodeposition. This release could be caused by either a reduction in microbial population size after the growth spurt, or because of a high rate of microbial turnover during the growth spurt.

Grazing-tolerance and N demand

Of all the resources required for plant growth, N is known to have one of the strongest effects on growth (Agren and Bosatta 1996; Lambers *et al.* 1998). There is a vast literature of both empirical and modelling studies supporting this pattern, many of which involve grasses (e.g. Garnier 1998). Lawn grasses can be expected to be more strongly affected by N than other (co-existing) grasses due to their tolerance of frequent defoliation during the growing season. Firstly, this defoliation would reduce retranslocation of N from old tissues to storage organs, and increase N losses. Secondly, if regrowth after defoliation is largely from residual (i.e. non-defoliated) leaf area, then rates of regrowth would be strongly dependent on net assimilation rate (NAR, the rate of growth per unit leaf area present), which is known to correlate strongly with plant N concentration (Lambers *et al.* 1998). It has therefore been suggested that increased N uptake after defoliation is an important aspect of grazing tolerance (Chapin III 1980; Jaramillo and Detling 1988).

While there is some evidence to substantiate the idea that grazing tolerance is dependant on high N supply, although it is mostly indirect. A number of community level studies show that a long history of grazing leads to an increase in above-ground tissue N concentrations (McNaughton 1990; Coughenour 1991). Similarly, in a pot experiment McNaughton (1992) found that clipping increased both leaf blade and sheath N concentrations for two species of Serengeti grasses, and found that the one dominant on heavily grazed sites had consistently higher N concentrations that the one from lightly grazed sites. Ruess *et al.* (1983) found that clipping led to faster rates of N uptake (per length root) for *Kyllinga nervosa*, a grazing-tolerant sedge from the Serengeti. Jaramillo and Detling (1988) found that defoliation increased N uptake (per unit root mass) and whole plant N concentrations for *Bouteloua gracilis*, a grazing-tolerant species from North American prairies. These results suggests that grazing-tolerant species are able to exploit an increase in N supply that accompanies grazing, but does not necessarily mean that they have a high N requirement.

Few experiments actually test the response of (regularly defoliated) grazing tolerant species to N supply. Almost all experimental work on plant responses to N involves species not known to be grazing tolerant, and does not include the influence of clipping. One exception is the study of Ruess *et al.* cited above did show that when clipped weekly, *K. nervosa* required a higher concentration of N to produce the same (residual) biomass as unclipped controls. The only other studies of this nature found in the literature were pot experiments performed by Georgiadis *et al.* (1989) and Hamilton III *et al.* (1998). They report that with frequent clipping, both *K. nervosa* and *Sporobolus kentrophyllus* (another lawn grass from the Serengeti) accumulated more biomass than unclipped controls, but only when N supply was high. Whether southern African species would show the same response is not clear. Only one paper was found which directly compared the growth of a local lawn species (Roux 1955), and it did not support the results cited above. In a sand culture experiment, Roux found that *Cynodon dactylon*, a widespread species common in heavily-grazed environments of southern Africa, showed toxicity to N at a lower level than a number of bunch grass species from highveld grasslands. This result should be treated with caution, however, as growth response was poorly measured, and N was supplied only as ammonium and at very high concentrations.

What does "response to N" mean ?

Before investigating whether lawn grasses of HUP do in fact show a large response to N, the exact meaning of "response to N" needs to be clarified. There are two reasons for this. Firstly, from an ecological perspective, "response" should incorporate reproduction as well as vegetative growth, as both processes influence the persistence of a plant in an ecosystem. Secondly, from a physiological perspective, vegetative growth response to "N" can be affected by:

- the chemical source that N is supplied as,
- spatio-temporal variation in the flux of N from the soil, and
- how N is used once it is acquired.

Vegetative growth responses

Vegetative growth can be reduced to two components. The first, purely physiological, is a measure of the amount of growth that a plant achieves per unit of N it has acquired (nitrogen-use efficiency). The second, partly ecological, is a measure of the ability of a plant to acquire N from its environment. These responses apply to the use and acquisition of any nutrient (or other resource) and are discussed as such.

Measuring nutrient-use efficiency

Originally **nutrient-use efficiency** was simply calculated as the rate of growth of a plant over a certain interval, divided by the nutrient concentration within the plant at the end of that interval (Lambers *et al.* 1998). However, nutrient concentrations within a plant can vary considerably due to the fact that supply of a nutrient from the environment often does not match the nutrient demand of a growing plant. And even small changes in internal concentrations can trigger changes in growth. Ingestad and Agren (1992) therefore proposed the use of **nutrient productivity**, the rate of growth per unit nutrient present in the plant per day, as a measure of efficiency with which plants use a nutrient. By maintaining a precise exponential supply of a limiting amount of N, Ingestad and Agren (1992) showed that constant internal concentrations can be maintained, and that this results in a very strong relationship between N concentration and relative growth rate. This has also been shown using a nutrient starvation technique (Burns 1992) and has subsequently been found for a wide range of grasses grown using more conventional methods (Garnier 1998).

However, plant growth is affected not only by the amount of a nutrient acquired, but also the duration that the nutrient is maintained (and used) within the plant, which is not taken into account in the concept of nutrient productivity. To incorporate the effects on nutrient turnover, nutrient-use efficiency was expanded to include the mean residence time of a nutrient within the plant, and is now defined as the product of nutrient productivity and "mean residence time" (Lambers *et al.* 1998). In other words, vegetative growth responses in response to nutrient acquisition is best measured as the rate of growth per unit nutrient acquired multiplied by the length of time that unit remains within the plant. However, this measure is rarely employed, probably because of the difficulty in measuring "mean residence time" (Agren and Bosatta 1996).

Measuring nutrient acquisition

The ability of a plant to acquire N (or any resource) from its environment is a far more complex topic, and has yet to be collapsed into a single measure such as nutrient-use efficiency. The complexity stems from the fact that:

1. N can be acquired in different chemical forms, and
2. the supply of nutrients from the soil shows both spatial and temporal variation, at a spatial scale smaller than the extent of a plant's root systems and a temporal scale shorter than the longevity of a plant.

The first point is dealt with in section 2. The second point is discussed below.

Spatio-temporal variation in N supply

Temporally, N supply can vary at scales ranging from months – net N mineralization can decline through a growing season in semi-arid systems (Mazzarino *et al.* 1998; Grant and Scholes 2002) - to days, due to uptake by roots of neighbouring plants (Grime 1994) or changes in rhizodeposition (Hamilton III and Frank 2001). Spatially, the distribution of organic N and N mineralization (as well as plant-available phosphorus and potassium) has shown to vary strongly horizontally, at distances at least as small as 12.5cm (Jackson and Caldwell 1993a, b), as well as vertically (inorganic N often decreases with depth - Stock and Lewis 1986; Evans and Ehleringer 1994; Mazzarino *et al.* 1998). Of particular interest to this study, is that grazing may modify, and amplify, such spatio-temporal variation. While Burke *et al.* (1999) found little effect of grazing on the spatial heterogeneity of organic soil N, dung and urine deposition does show spatially aggregation (pers. obs., Shiyomi 2000 cites Hakamata 1999) which might create local hotspots of N mineralization (regardless of organic pool sizes). Furthermore, Augustine and Frank (2001) found that grazing increased (horizontal) spatial heterogeneity in N mineralization at scales fine enough to be relevant to individual plants, apparently as a result of changes in the distribution of litter inputs.

The relevance of such spatio-temporal variation to plant growth has only recently been demonstrated experimentally. When a nutrient is supplied uniformly across the entire root system, plants vary in their rate of acquisition of it as a result of differences in the area of the root system (relative to plant size) and / or differences in the rate the rate of uptake per unit area of root (specific uptake rate). When a nutrient is supplied non-uniformly, acquisition can be further influenced by disproportionate changes in either the area or specific uptake rate of any part of root system occurring within patches (spatial or temporal) of high nutrient supply. This is referred to hereafter as **foraging**, following the definition of Hutchings and de Kroon (1994 , p150): '… the process whereby an organism searches, or ramifies within its habitat, which enhances its acquisition of essential resources'. There is growing evidence that plants are able to forage for nutrients in both space and time.

"Spatial foraging"

Empirical evidence for the ability of plants to proliferate roots in patches of high nutrient availability dates back to the 1920's and includes a vast literature (reviewd by Robinson 1994). However, many of these studies, as well as more recent ones (Burns 1992; Fransen *et al.* 1998; Hodge *et al.* 1998; Fransen and de Kroon 2001) found no increase in growth as a result of such proliferation. In addition, a model of root uptake by Robinson (1996) indicated no benefit to localized root proliferation for the uptake of nitrate, due to the high mobility of this ion (although there was a benefit for the phosphate uptake). However, amongst more recent studies (cited below), many provide evidence for enhanced nutrient uptake and / or growth in response to a patchy rather than uniform supply of the same overall amount of nutrients. This is achieved through either a change in root architecture (Hodge *et al.* 1999b), the proliferation of lateral roots Cui and Caldwell 1998; Hodge *et al.* 1999a; Robinson *et al.* 1999), or a combination of proliferation and increased specific uptake (Crick and Grime 1987; Caldwell *et al.* 1991b; van Vuuren *et al.*; Fransen *et al.* 1999; Fransen and de Kroon 2001). Finally, a simulation model by Jackson and Caldwell (1996) indicates that that through root proliferation and increased specific uptake, the acquisition of both nitrate and phosphate ions can be higher when these nutrients are supplied in patches.

Temporal "foraging"

Few studies have investigated the effect of temporal variation in nutrient supply *per se*, although most of the studies cited above incorporate a degree of temporal variation as nutrient-enriched patches are not renewed. Regarding the effects of temporal variation alone (with nutrients supplied uniformly in space) Campbell and Grime (1989) report increased growth in response to discrete pulses of nutrients for two grass species.

Clonal plants, such as grasses, have the potential to respond to spatio-temporal variation in nutrient supply by modifying not only their roots, but also the placement of those roots in space (Schmid 1986) and time. This could be achieved by altering ramet branching, inter-ramet distances, ramet longevity and the physiological integration of ramets (Caraco and Kelly 1991; Cain 1994; de Kroon and Hutchings 1995). However no clear evidence of such changes occurring in response to a patchy nutrient supply, and leading to increased growth, were found in the literature.

Reproduction

A final aspect to consider regarding "response" is that of seed production and germination. A plant's vegetative growth response may be of little ecological significance if it does not result in increased reproduction. While seed production is often correlated with vegetative growth (Chapin III 1980), seed germination requirements may not necessarily reflect vegetative growth responses. If a plant has relatively fast growth at low N supply, but requires a large N supply for germination, then vegetative growth responses would not explain the absence of the species from "N poor" habitats. N supply has been shown to affect germination rates of a number of herbaceous species (Wamelink; Young *et al.* 1999). In addition, growth immediately following germination may be different from growth at a larger size, making seed size important (Fichtner and Schulze 1992; Elberse and Berendse 1993). As growth of seedlings is usually exponential, it is strongly influenced by initial mass (i.e. seed mass). This could be of ecological significance if a potential to monopolize access to another resource exists. Thus a plant with a slow (adult) growth rate at a given supply of N, but with a large seed size, could initially grow larger than a faster-growing, small-seeded species, monopolize access to another potentially limiting resource and exclude the vegetatively "fast growing" species.

To conclude, the literature provides good evidence that grazing accelerates the supply of inorganic N, as well as some preliminary evidence that grazing-tolerant species show a strong response to a high supply of this N. However, "response to N" is complex concept as it encapsulates both vegetative growth (including nutrient use efficiency) and reproduction, and the effects of the chemical source, rate and spatio-temporal variation of N supply. The remainder of this chapter focuses on vegetative growth, rather than reproduction. Section 2 investigates the effects of the chemical source of N on lawn grass growth, while section 3 addresses the importance of the rate of supply on growth and makes some predictions regarding the effects of spatio-temporal variation in supply on the growth of lawn grasses.

SECTION 2

NITROGEN SOURCE

INTRODUCTION

This section investigates the effect of the source of mineral N on the growth of three lawn grasses. The aim is to both identify an appropriate N source for testing the effect of the rate of N supply on growth (section 3), and to test whether lawn grasses show a common preference for a particular source of N.

Why N source?

Plants have long been known to utilise two sources of inorganic N - **ammonium** and **nitrate** (Clarkson 1985; Lewis 1986; Lambers *et al.* 1998). More recently it has also been discovered that many species utilise **organic N** (Lipson and Nasholm 2001). At a regional scale, there is a coarse pattern of differential use of these source: many arctic and temperate species show higher uptake of, and growth on, ammonium or organic N (in the form of amino acids - Lipson and Nasholm 2001), while subtropical and tropical species, which grow in environments where temperature and moisture are less likely to inhibit N mineralization and nitrification, show a greater ability to utilize nitrate (Lewis 1986; Lambers *et al.* 1998). However, it has been suggested that co-existing species have evolved adaptations to exploit different sources of N.

Based on differences in rates of nitrate reductase activity for species from a *Banksia* woodland in SW Australia, Stewart *et al.* (1993) suggest that herbaceous species use predominantly nitrate and woody species ammonium (nitrate reductase, as well as nitrite reductase, are the enzymes used to reduce nitrate to ammonium, which is the only form on N which plants can assimilate – the activity of this enzyme is therefore considered a measure of a species ability to utilize nitrate). Differences in the relative abundance of foliar ^{15}N has led to suggestions that species of different growth forms (trees, shrubs, herbaceous species) of Alaskan tundra (Schulze *et al.* 1994) and boreal ecosystems (Nadelhoffer *et al.* 1996) utilize different sources of N. Similarly, it has been suggested that grass species dominating different stages of allogenic succession are adapted to exploit different sources of inorganic N (see below).

Which source?

For this study, the hypothesis tested is that lawn grasses share a common preference (in terms of growth rates) for the nitrate source of N. This is derived from two sources within a much broader literature on N source.

Grazing and nitrification

While the supply of N has long been thought to drive successional changes in the highveld grasslands of southern African, it has been argued that N source, rather than amount, is the key factor (Wiltshire 1973). This stems from two studies (Stivens 1957; Grossman and Cresswell 1974) which report that concentrations of soil nitrate fall far more than ammonium as grassland succession proceeds. As this succession often occurred subsequent to the cessation of intensive grazing, this pattern could be interpreted as evidence that grazing facilitates relatively higher rates of nitrification (the microbiological process by which ammonium is converted to nitrate). Further evidence comes from a ^{15}N natural abundance experiment by Frank and Evans(1997). In Yellowstone National Park (North America), Frank and Evans found that grass leaf ^{15}N was significantly less abundant (relative to ^{14}N) on heavily grazed sites than inside grazing exclosures. As nitrification discriminates against the ^{15}N isotope (relative to ^{14}N), this could indicate that the supply of nitrate is relatively higher at the grazed sites. Finally, comparing soils inside and outside a grazing exclosure over 40 years old Dormaar and Willms (1998), found that with grazing soil nitrate concentration increased more so than ammonium concentration.

Growth responses of local grasses

In arguing that grassland succession is driven by a reduced supply of nitrate, Wiltshire (1973) proposed that "climax" species (species which dominate in lightly or ungrazed communities which have not been ploughed) have a relatively high requirement for ammonium. Conversely "seral" species (those which dominate "overgrazed" communities and / or severely disturbed areas, and therefore a category which includes lawn grasses) would have a relatively high requirement for nitrate. In a review of a pot experiments, Wiltshire reports that the biomass accumulation of a range of southern African grassland species (all bunch grasses) was greater with ammonium N, and that this effect was greater for climax species (with yields over 4 times greater for some species). Grossman and Cresswell (1974) found that three species (*Eragrostis curvula, Hyparrhenia hirta* and *Themeda triandra* – climax and sub-climax bunch grasses) produced more biomass when supplied with 200ppm ammonium rather than nitrate. Finally, Bate and Heelas (1975) found that *Sporobolus pyramidalis* (a species common on grazing lawns in HUP), had 4-fold greater nitrate reductase activity than *Hyparrhenia filipendula* (a climax species), as well as higher leaf nitrate concentrations, both of which suggest a greater ability to acquire nitrate. Finally, Ruess *et al.* (1983) found that the grazing-tolerant sedge from the Serengeti, *Kyllinga nervosa*, produced more biomass when N was supplied as nitrate rather than ammonium, in solution culture (although this effect was reversed at the lowest rate of N supply).

Contrary to the hypothesis proposed above, mechanistic biochemical models of N assimilation predict that ammonium assimilation requires less energy, and therefore allows for faster growth rates, and more efficient use of light, water and certain cations for all plants (Raven *et al.* 1993). However, experimentation has shown that this is often not the case (Raven *et al.* 1992; Raven *et al.*; Lambers *et al.* 1998). Crop species in particular show faster growth and greater water use efficiency when N is supplied as nitrate. A recent cost-analysis model by Zerihun *et al.* (1998) provides a potential explanation for this. These authors agree that nitrate assimilation does cost more (in terms of glucose equivalents) because of the energy required for the reduction of N and for the production of acids needed to neutralize the OH^- produced by this reduction. However, their model shows that the cost of N assimilation is small relative to the overall costs of protein synthesis, when there is a high turnover of proteins (i.e. the initial cost of amino acid synthesis is likely to be small relative to the cost of continual re-use of those amino acids for protein production). Furthermore, the cost of pH regulation may be off-set by the benefit of osmotic solute generation. Malic acid which is thought to be used to neutralize OH^- also plays an important role in leaf-area expansion. Thus the short-term costs of nitrate assimilation may be very small when considered against all the growth costs that accrue over a longer time period. Finally, under natural conditions the lower mobility of ammonium through the soil means that even if nitrification of ammonium is limited, nitrate may still be more available as a source of N for plants (Raven *et al.* 1992).

In designing an experiment to test the response of lawn grasses to N source, incorporating all the complexity involved in comparing growth response (section 1) was not attempted. Rather, plants were simply grown in sand culture and provided with regular nutrient renewals, and growth measured simply as the vegetative relative growth rate. It can only be assumed the results apply to reproduction as well as vegetative growth, and when N supply is less uniform.

METHODS

Plant material

Four perennial species, all common in HUP, were used. *Digitaria longiflora* (Retz.) Pers. and *Dactyloctenium australe* Steud., are dominant on grazing lawns in the mesic areas of the park, where the mean annual rainfall is approximately 800mm (pers. obs., Page and Walker 1978). The former only occurs in the open, while the latter is more common on lawns under tree canopies and also occurs in more arid areas. The other two species, *Urochloa mossambicensis* (Hack.) Dandy and *Panicum maximum* Jacq. are most common in the semi-arid areas of HUP (mean annual rainfall of approximately 650mm). The former is most common on unshaded grazing lawns, where it is often the dominant species. The latter, although occasionally found on lawns, is more common on lightly grazed areas, and usually only dominant under tree canopies. These species are referred to by their generic names hereafter. *Digitaria* and *Dactyloctenium* are strongly stoloniferous species. *Urochloa* can grow vertically as bunch grass (up to 1m tall - van Oudtshoorn 1999) but is stoloniferous when grazed. *Panicum* is the largest of the four, being a bunch grass up to 2m tall, although it may produce stolons when heavily grazed (pers. obs.). All four species have a C_4 photosynthetic pathway, and *Panicum maximum* has the PCK sub pathway (Baruch and Gomez 1996). Sub pathways of the other three species are unknown, but other members of the genus *Digitaria* are NADP-ME, and other *Urochloa* species are PCK (Gibbs Russel *et al.* 1990).

Plants were collected from HUP and grown in a glasshouse at the University of Cape Town (UCT) in sand culture. For each species at least one individual was collected from at least two locations. These locations were considered to be too far apart for cross pollination between the populations to occur and differed in respect to soil type or mean annual rainfall, or both. Before the experiment, clones (each a single ramet) were produced in the glasshouse by separating the rooted nodes of stolons or cutting rooted culms from crowns. For each treatment level in each experiment, equal numbers of clones were produced from two individuals collected from two different locations. Clones were made as small as possible, and each ramet consisted of a single rooted node, 1-3 nodal roots and 1-2 adult leaves.

Experimental design

Due to limited growth chamber space, all four species could not be tested in the same experiment. Instead, three successive experiments were conducted. The two smaller species, *Digitaria* and *Dactyloctenium* were grown together in the first experiment while the two larger species were grown separately in two further experiments. The latter two experiments were performed simultaneously, in two different phytotrons. Five replicates were used in each experiment. For *Urochloa* and *Panicum* there was one plant per pot. For *Digitaria* and *Dactyloctenium* there were two plants per pot, one of each species (**Fig. 2.1**). Plants were rinsed, blotted with tissue paper, stripped of any dead roots and leaves, and weighed before planting.

Growth conditions

Plastic rectangular pots (45cm x 30cm x 15cm) each holding approximately 20 litres of coarse sand (Consol Filter Sand) were used for all experiments. The two phytotrons used provided a light intensity of approximately 900 μmol. m^{-2} s^{-1} PAR (measured with a Skye light meter) at a height of 2cm above the sand. Temperature was set to 30/20°C (light/dark) and lights to: 9hr dark, 1hr step up, 13hr full intensity, 1 hr step down. Humidity could not be regulated and tracked outdoor levels.

The pots were watered once every three days. This involved saturating each pot with deionized water, in an attempt to displace any remaining nutrient solution from the previous watering from the pot, neutralize pH changes and minimize microbial build-up. After saturation, 500ml of nutrient solution was added. Nutrients other than N were supplied as Long Ashton nutrient solution, which contained the following concentrations of macronutrients (mM): 4 K^+, 4 Ca^{2+}, 3.5 SO_4^{2-}, 2.1 Na^+, 1.5 Mg^{2+} and 1.3 PO_4^{3-}; and micronutrients (:M): 8000 Cl^+; 140 BO_3^{3-}, 21 Mn^{2+}, 2.3 Zn^{2+}, 0.96 Cu^{2+}, 0.31 MoO_4^{2-} and 97 Fe^{2+} (as EDTA). N was supplied as either 2.9 mM of KNO_3 (**nitrate treatment**), 2.9 mM NH_4Cl (**ammonium treatment**) or 1.45 mM KNO_3 plus 1.45 mM NH_4Cl (**mix treatment**).

Figure 2.1. The sand culture experiment carried out within a phytotron at University of Cape Town. Pots contain two to three individuals lawn grasses (*Digitaria longiflora* or *Dactyloctenium australe* or both), which were supplied with either nitrate, ammonium or a combination as a source of nitrogen. The photograph was taken shortly before harvest, after about a month of growth.

While sand culture is more practical than solution culture, pH cannot be accurately controlled. When N is assimilated as ammonium, pH falls due to the release of a proton for each NH_4^+ ion assimilated, but rises with nitrate assimilation due to the production of an OH^- ion when an ion of NO_3^- is reduced to NH_4^+ (Lambers *et al.* 1998). In order to test whether large changes in pH occurred, samples of nutrient solution were taken towards the end of each experiment and pH measured (WTW ph330 meter). The first approximately 100ml of solution which drained from a pot when saturated with deionized water was used.

Defoliation

To simulate grazing under natural conditions, plants were clipped to a height of 3cm on the second day of each experiment (after morphometric measurements had been made), and every 28 days thereafter for the duration of the growth period. Stolons were removed completely if above this height at any point along their length. However, if a stolon was rooted beyond the point of clipping then any part of this distal portion below 2cm was not removed.

Harvest

Plants were harvested once the area of the pot had been filled by the above-ground parts (between 40 and 60 days after planting). Plants were separated into root, crown (including any rooted nodes), upright culms, horizontal culms, stolons (horizontal culms with at least one rooted node) and leaves (blades plus sheaths). Prior to this, plants were clipped in order to estimate the proportion of each above-ground part lost to defoliation. Clipped and harvested plant matter was dried at 60°C until at constant mass (approximately 72 hours).

Response variables

Growth was calculated as the mean relative growth rate over the entire duration of the experiment (RGR):

$$\mathbf{RGR} = (\ln W_2 / W_1)/\Delta t$$

where, W_1 and W_2 are the fresh weights of an individual plant at the beginning and the end of the experiment respectively, and Δt is the duration of the growth period. This model assumes that growth occurred at a constant exponential rate over the duration of the experiment, and that plant moisture content did not change. Space limitations excluded the option of multiple harvests to test whether these assumptions were correct. As growth period durations varied greatly, the simpler analytical approach of comparing final biomass only is not applicable. Allocation to root was calculated as root mass fraction (**RMF**), the fraction of whole plant dry weight made up by root dry weight.

Statistical analysis

Separate factorial ANOVA models, using the GLM module of STATISTICA 6.0 (StatSoft 2001), were used to test for differences in RGR and RMF between treatments and species. No transformation was required to meet the assumptions of homogeneity of variance and normality for RGR. For RMF there were significant differences in variance between groups, but this was not overcome by standard ln transformation. However, since variances did not appear to correlate with the means, this is not a serious violation of the ANOVA assumptions (StatSoft 2001), and results are still presented. Post hoc analyses were performed with the Least Significant Difference test.

RESULTS

pH

pH dropped, from an initial value of 6.8 in the nutrient solution, for all treatments (**Fig. 2.2**). Decreases were larger for the mix and ammonium treatments, but did not appear to differ between these two treatments for any species. For the nitrate treatment, decreases were greater for *Urochloa* and *Panicum*. As a result the difference in pH between the nitrate treatment and the other treatments was far greater for *Dactyloctenium* and *Digitaria*.

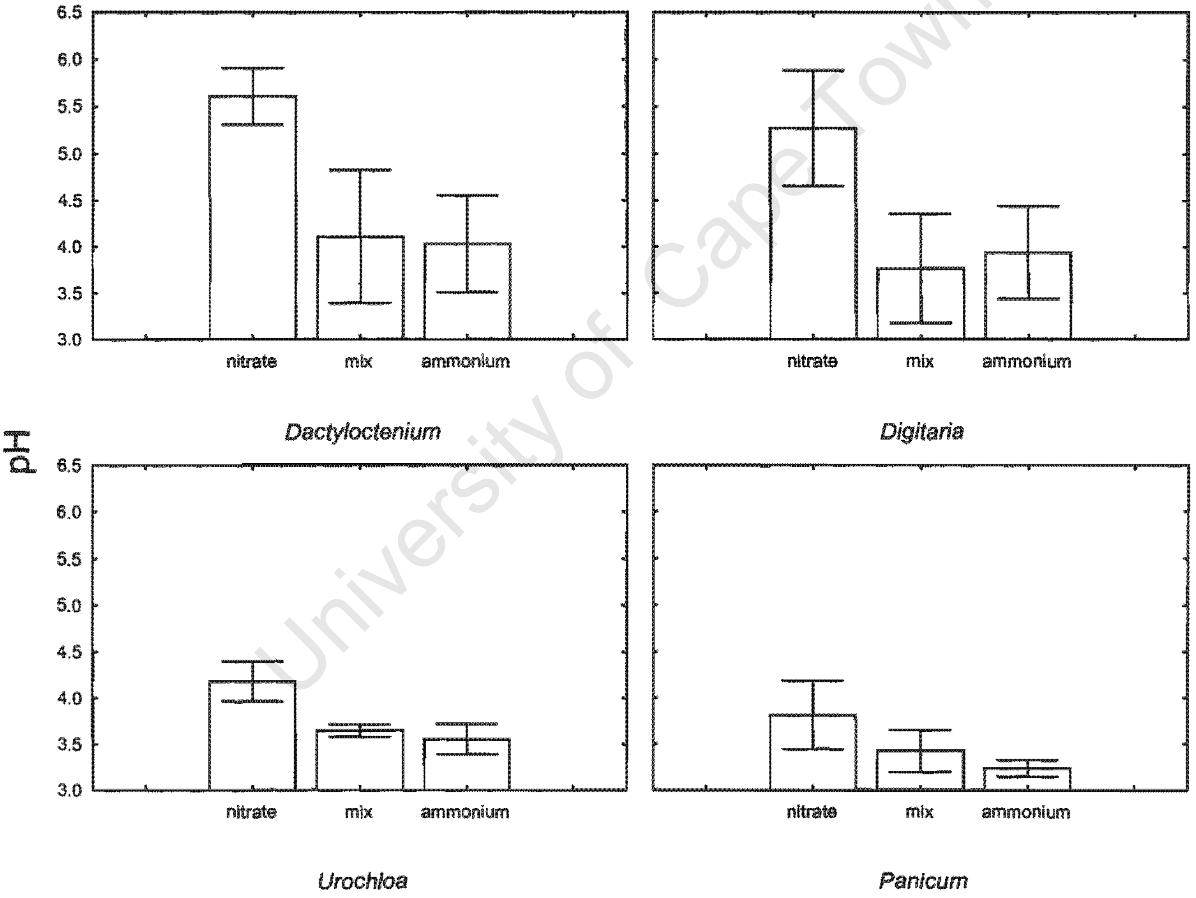


Figure 2.2. The effect of inorganic N source on mean pH of depleted nutrient solution drained from the pots of the three lawn grass species and one bunch grass species (*Panicum maximum*) grown in a sand culture. The initial pH of the nutrient solution added to pots, every 3 days, was 6.8. Whiskers show 95% confidence intervals.

Growth

RGR

RGR was barely affected by N source (**Fig. 2.3A**) and the factorial ANOVA model provided a poor fit to the data (**Table 2.1**). Neither species, treatment or the "species x treatment" interaction was significant, i.e. RGR was not significantly different for any treatment level, whether analysed for all species combined or for each species separately. However, a Student's t test revealed a significant difference between the nitrate and ammonium treatment RGR for *Urochloa* alone, with faster growth for the nitrate source of N (df = 8, t = 2.77, p = 0.024).

Allocation to root

Patterns in RMF reflected those in RGR. The factorial ANOVA model did provide a significant fit to the RMF data from all three experiments, but there was no significant "species x treatment" interaction (**Table 2.1**). Post-hoc analysis indicated a significantly higher mean RMF for the nitrate treatment than the ammonium treatment for *Dactyloctenium*. For *Digitaria* and *Urochloa*, RMF was significantly higher for the nitrate treatment than the mix treatment (**Fig. 2.3B**). As a result, when comparing between treatments, RMF (averaged across all species) was higher for the nitrate treatment, although not significantly so. Comparing between species, RMF (averaged across all treatments) was significantly affected, with all pair-wise comparisons being significant except that between *Dactyloctenium* and *Urochloa*.

Table 2.1. Main and interaction effects for the GLM factorial ANOVA models for the relative growth rate (RGR) and root mass fraction (RMF) of the three lawn grass species, *Digitaria, Dactyloctenium* and *Urochloa*, and one bunch grass species, *Panicum* grown in sand culture on either ammonium, nitrate or a combination of both sources of nitrogen. The model was not significant for RGR ($r^2 = 0.15$; $F_{11,47} = 1.95$; $p = 0.057$) but was for RMF ($r^2 = 0.52$; $F_{11,47} = 6.70$; $p < 0.001$). Note that F values for the effects of the RGR model were not calculated as the model was not significant. SS = sum of squares, df = degrees of freedom.

	RGR					**RMF**				
	Effect	**SS**	**df**	**F**	**p**	**Effect**	**SS**	**df**	**F**	**p**
Intercept	Fixed	0.159	1	176	0.0009	Fixed	8.14	1	115	0.0017
Species	Random	0.0027	3			**Random**	**0.212**	**3**	**12.3**	**0.0057**
Treatment	Fixed	0.0005	2			Fixed	0.37	2	3.18	0.11
Species x Treatment	Random	0.0007	6			Random	0.035	6	1.48	0.21
Error		0.0085	47				0.183	47		

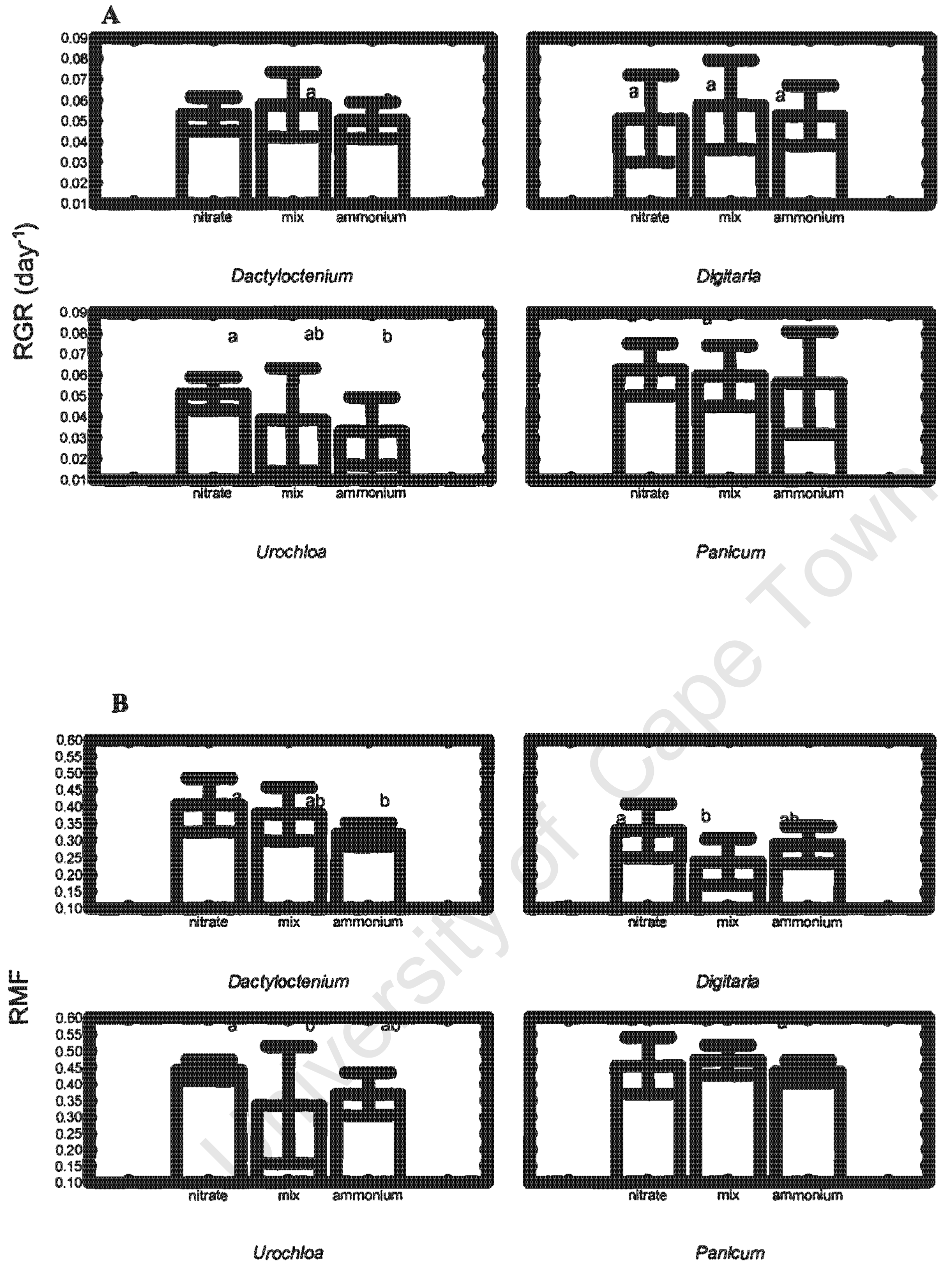


Figure. 2.3. The effect of inorganic N source on **A**. the mean RGR, and **B**. the mean root mass fraction (RMF) for the three lawn grass species and one bunch grass species (*Panicum*) grown in a sand culture. For each variable and each species, the same letters show non-significant differences according to a LSD test of a factorial ANOVA. Whiskers show 95% confidence intervals.

DISCUSSION

While *Urochloa* showed slightly faster growth on nitrate, the RGR values for *Digitaria* and *Dactyloctenium* provide no support for the hypothesis that lawn grasses share a preference for the nitrate source of N. Before discussing this within the context of other research on N source, the validity of this pattern is considered, as there are a number of factors which may have obscured the effect of N source on growth.

Potential experimental errors

Estimation of RGR

Plant growth is difficult to measure because biomass accumulates in a non-linear fashion through time. This problem is discussed further in the following chapter, but it should be pointed out that the shape of growth responses would be made even more complex by clipping. And as regular harvests were not performed, an accurate description of biomass changes through time could not be made. Instead, it can only be assumed that all plants had a constant exponential rate of growth over the entire growth duration, or at least all deviated from a constant rate equally.

Limited nutrient uptake

pH

Unregulated changes in pH may have confounded the results of this experiment, as is the case with many N source experiments (Lambers *et al.* 1998). The greater decrease of pH on the mix and ammonium treatments may have inhibited the uptake of other nutrients, in that additional protons can displace nutrient cations adsorped to sand particles, resulting in the leaching (Lambers *et al.* 1998). However, this is unlikely as little adsorption would have occurred on the coarse sand particles used anyway. In addition, as watering was immediately followed by nutrient renewal, any leaching would have been of little consequence.

Nutrient supply

The rate of supply of nutrients other than N may have limited growth, regardless of pH. The low RGR values across all treatments (far lower than the maximum RGR values estimated for three of these species in the following section) certainly suggest this. Therefore the plants may have been prevented from exploiting a preferred source of N because of a limited supply of another nutrient. It is unlikely that the supply of N itself was too low to elicit a response, as several other 'N source' experiments show that treatment effects are stronger at lower N supply. In the solution culture experiment of Ruess *et al.* (1983), also involving a lawn grass species and frequent clipping, the largest differences in final biomass between plants supplied with either nitrate or ammonium was found at the lowest supply rate of N (0.1 :M). In a sand culture experiment, Wolfson (1988) found significant effects of N source for *Digitaria eriantha* at the lowest N supply treatment of 50 mg N litre^{-1} supplied twice weekly (assuming nutrients solutions saturated pots in both experiments, this is the equivalent of approximately 1.1 mM N day^{-1}, as compared to 9.6mM N day^{-1} in this experiment). Finally, for the grass species which showed far greater growth on ammonium, Wiltshire (1973) reports leaf N concentrations of only about 1%, which probably indicates N deficiency.

Growth duration

The duration of the growth period may have been too short for the differences in N acquired to translate into differences in growth. Since many *Digitaria* and *Urochloa* plants produced inflorescences before harvest, it seems unlikely that some form of ontogenetic drift (Coleman *et al.* 1994) prevented differences from establishing within the experimental period. However, it is possible that with more growth, tissue concentrations of ammonium could have accumulated to levels high enough to reduce growth. For example, for *D. eriantha* growth on 50mg N litre^{-1} nitrate only began to exceed that on ammonium after 2 months (and when plants were clipped, this pattern took a further 2 months to re-establish - Wolfson, 1988). However, plants were supplied with far less light in that study, and RGR values were considerably lower (approximately 0.0035 g^{-1} g^{-1} day).

The significance of a lack of preference for N source

Assuming that the RGR patterns observed are accurate, the results suggest that lawn grasses do not share a common preference for nitrate, although this may be the case for some of the species. If anything, there appears to be a division between the semi-arid species (*Urochloa* and *Panicum)* and the mesic species (*Digitaria* and *Dactyloctenium*), rather than between lawn and bunch grasses. As mentioned in the *Introduction*, evidence for the hypothesis that they would show a preference for nitrate was derived from only a part of the literature on N source. Other studies, which suggest that the observed lack of preference is valid, are discussed below.

Growth responses of local species to N source are contradictory

Bunch grasses

There are some local studies which indicate that bunch grasses do not in fact grow faster on ammonium. Based on measures of CO_2 compensation point (at the leaf level) Grossman and Cresswell (1974), suggested that the secondary and climax species they studied were more sensitive than the seral species to high levels of ammonium because of increased photorespiration. And while all three species showed higher growth on ammonium at 200ppm, the secondary and climax species showed decreased biomass production, or even death, at 400ppm ammonium (approximately 7mM N in solution culture). However, increased biomass production was maintained at this level by the seral species. This is somewhat supported by an older sand culture experiment (Roux 1954) which showed that height increase was more sensitive to increasing ammonium for a climax species (*Trachypogon plurosus*) than for a "sub-climax" species (*Eragrostis curvula*). Tew *et al.* (1974) proposed that climax species are sensitive to a high supply of ammonium because they lack an ability to regulate high internal concentrations of ammonium. They suggested that only C_4 species which produce aspartate as the first product of CO_2 assimilation (now classified as the NAD-ME or PCK C4 biochemical subpathways – Lambers *et al.* 1998) are able to tolerate high levels of ammonium (although they did not suggest a physiological reason for this). Interestingly, their observation that the climax species which they studied (*Themeda triandra*) was a malate producer (now classified as NADP-ME) appears to be relevant for climax species in general, as most climax species are of the tribe Andropogoneae and almost all andropogonoids are NADP-ME C_4 species (Sage *et al.* 1999). The proposed pattern of bunch grasses showing a preference for the ammonium source of N is therefore doubtful.

Lawn grasses

Similarly, there is evidence that lawn species sometimes show a preference for ammonium rather than nitrate. A solution culture experiment (performed in the same phytotron as the one used in this study) found that *Cynodon dactylon* (the lawn grass of remarkably similar morphology to *Digitaria)* produced more biomass when supplied with ammonium than with nitrate (Mafa 1999). Also, *Kyllinga nervosa*, the grazing lawn species from Serengeti, had higher leaf concentrations of nutrients (except calcium and magnesium) when grown on ammonium as opposed to nitrate (Ruess 1984), and actually produced more on ammonium, at a low rate of N supply (Ruess *et al.* 1983).

A preference for ammonium?

In this study, when species were supplied with both ammonium and nitrate (mix treatment), all appear to have taken up ammonium preferentially. This is indicated by firstly, the fall in pH on the mix treatments to levels as low as on the ammonium treatments. Secondly, RMF on the mix treatment was not significantly higher than on the ammonium treatment. As reduced allocation to root is common when ammonium is the sole source of N (Guo *et al.* 2002), this suggests that more ammonium was taken up than nitrate. As diffusion rates of ammonium and nitrate in solution are similar (Raven *et al.* 1992), this in turn suggests preferential uptake of ammonium. In addition, faster rates of ammonium uptake were found by Ruess *et al.* (1983), where N uptake was measured directly with the use of specific ion electrodes. However, this does not necessarily mean that lawn grasses are adapted to an ammonium source of N, as preferential uptake of ammonium may be a general plant response. This has been found for other grass (non lawn grass) species, such as rice and maize (Taylor and Bloom 1998), and there is evidence for a range of species that ammonium uptake inhibits nitrate uptake when the two are supplied together (Raven *et al.* 1992).

The effect of grazing on N cycling is not clear

Working in the same ecosystem as Frank and Evans (1997), Frank *et al* (2000) measured rates of net mineralization, nitrification and denitrification directly (using resin bags) and found that while grazing stimulated both net mineralization and nitrification, the increase was greater for the former. This suggests that with grazing, ammonium would become relatively more available than nitrate. Furthermore, Bate and Heelas (1975) found higher concentrations of soil nitrate at their "undisturbed" site, dominated by *Hyparrhenia filipendula* (a grazing-intolerant bunch grass) than at their "disturbed site", dominated by *Sporobolus pyramidalis* (a species known to increase with grazing - van Oudtshoorn 1999). More research of soil N cycling is needed to clarify the effect of grazing on the relative supply rates of ammonium and nitrate.

Organic N

Finally, the above discussion assumes that lawn (and bunch) grasses use only inorganic N. However, considering the recent proliferation of studies showing that a wide variety of plants utilize organic N, the possibility that these grasses utilize this source of N deserves attention. While the original conclusive evidence of organic N uptake comes from tundra (Chapin III *et al.* 1993; Schimel and Chapin III 1996) and boreal ecosystems (Nasholm *et al.* 1998), more recent work provides evidence of organic N use in ecosystems as diverse as deserts, including grasslands (Colorado steppe) and savannas (Australian semi-arid and tropical woodlands - Lipson and Nasholm 2001). Many studies provide direct evidence of grasses taking up organic N. These include *in situ* experiments, in which mutualisms with arbuscular mycorrhizae appear to facilitate uptake (Nasholm *et al.* 1998), and solution culture experiments which exclude mycorrhizae: Chapin III *et al.* (1993) and Lipson and Nasholm (2001) for *Zea mays* and *Hordeum vulgare*, and Henry and Jefferies (2002) for *Puccinellia phryganodes,* a grazing-tolerant salt marsh grass.

In their review, Lipson and Nasholm (2001) argue that use of organic N is most likely to be of significance in ecosystems where microbial biomass undergoes large seasonal fluctuations. This is because the major source of organic N for plants is thought to be amino acids "leaked" from dead microbes. This may well be the case for the semi-arid grasslands and savannas of southern Africa which all experience regular wetting and drying of the soil profile during the growing season, as a result of long intervals between rainfall events. If microbes die-off at a soil moisture potential above that at which plant nutrient uptake ceases, then it is likely that local grass species have evolved to exploit a potentially large supply of amino acids. Furthermore, grazing could exaggerate organic N supply in certain environments. This is because grazing can lead to an accumulation of salts such as sodium in the soil (McNaughton *et al.* 1997b) and high levels of salinity can reduce rates of both urea hydrolysis and nitrification (Wilson *et al.* 1999). Therefore high levels of grazing could increase the availability of organic N indirectly, even when soil moisture levels are high enough for microbial growth. Whether either of these processes do in fact occur remains to be seen.

Conclusion

One aim of this experiment was achieved, in that it was found that supplying N as ammonium nitrate would produce as much growth as either ammonium or nitrate alone. However, it is not clear from the results whether lawn grasses share a common growth response to a particular source of inorganic N. When considering the results in the context of other N source research, there is little reason to believe that lawn grasses have evolved to exploit one particular source of N. Rather it likely that different lawn species exploit different N sources, as with bunch grasses. Future research also needs to consider the possibility that lawn grasses utilize organic N.

SECTION 3

RATE OF NITROGEN SUPPLY

INTRODUCTION

This section reports on the results of a series of experiments designed to determine the effect of the rate of supply of N on the growth of four species of lawn grass from HUP. The hypothesis tested is that the lawn grasses share a common, large response to N supply. Again, the experiments do not incorporate all the complexity of 'response' to N described in section 1. Only the most simple aspects of 'response to N' are tested: vegetative growth response to a spatiotemporally uniform supply of N. In an attempt to gain some insight into how the species involved differ in respect to the other aspects of 'N response', allocation patterns were also measured and analysed. Part 1 of this section considers the direct, vegetative growth responses (in terms of relative growth rate), part 2 considers the significance of the allocation patterns observed, and part 3 discusses factors which need to be taken into account when extrapolating the results of experiments conducted under controlled conditions. While both parts1 and 2 present and discuss results from the same experiments, the *Methods* section has been split between the two parts to separate the description of the response variables relevant to each part.

Experimental rationale

Measuring vegetative growth responses to a uniform supply of N may bring theoretical simplicity but still poses technical problems:

1. The effect of the supply of other resources should not interfere, or should affect all species equally. The simplest means of achieving this is to supply all other resources at a non-limiting, but also non-toxic, rate. Other factors which can affect growth, such as temperature and pH, also need to be maintained within a non-limiting range.
2. Continuous uptake by exponentially growing plants makes supplying the nutrient uniformly very difficult.

The use of solution culture minimizes these problems. Ideally one would use a either a flowing solution with very frequent renewal of nutrients (Asher and Edwards 1983), or the aeroponics technique described by Ingestad (1982). However, both these techniques are expensive and difficult to apply when wanting to include many replicates. The relative addition rate technique described by Ingestad (1982) allows for more accurate control of plant uptake, and reasonably accurate results have been achieved by employing this technique using the traditional large-volume, infrequent renewal solution method (Boot *et al.* 1990a; Stadt *et al.* 1992). However, this still requires estimates of initial plant N concentrations, appropriate relative addition rates, and multiple harvests, all of which would be very time consuming when wanting to test a large number of species. Therefore, the traditional 'single dose' method using a large volume of nutrient solution, and weekly renewal, was chosen. As this method has the problem of N solution concentrations dropping between renewals (particularly as plants grow larger) concentration differences between treatment levels were kept large (3 to 4 fold). Note that depletion between renewals is not likely to be a problem for other nutrients, in that they can be supplied at a concentration well above that thought to be optimal.

PART 1

RELATIVE GROWTH RATE

METHOD

Experimental design

Four grazing lawn species were used, but due to space limitations an adequate number of replicates of all four could not be grown together at one time. Therefore four separate experiments were performed consecutively, one for each species. These experiments were conducted as much as possible in the same manner and the methods described apply to all experiments, unless otherwise stated. Space limitations precluded adequate replication for multiple harvesting, while the stoloniferous habit of three of the species used made it all but impossible for repeated weighing of plants as they grew. N was supplied on a gradient of at least 5 levels - the highest level was used to test for toxic effects of a high N concentration.

Plant material

Four perennial species which are dominant on grazing lawns in HUP, and observed to be tolerant of frequent grazing, were used: *Digitaria longiflora* (Retz.) Pers., *Dactyloctenium australe* Steud., *Urochloa mossambicensis* (Hack.) Dandy and *Sporobolus nitens* Stent (referred to by their generic names hereafter). The first three were described in section 2. *Sporobolus,* like *Urochloa*, is dominant on unshaded lawns in the semi-arid parts of HUP, although it forms large patches on unshaded lawns in mesic areas as well. It is a very short, rhizomatous species that does not produce culms other than flowering culms. Like the other species, it has a C_4 photosynthetic pathway. The subpathway is unknown but other members of the genus are PCK or NAD-ME (Gibbs Russel *et al.* 1990). Collection and cloning of plant material was described in section 2.

Growth conditions

Clones were placed into a 2mM $CaSO_4$ solution (*Digitaria*, *Urochloa*, *Dactyloctenium*) or deionised water (*Sporobolus*) for 18-24hrs. They were then moved into a quarter or half-strength Long Ashton solution (Hewitt 1966) in a phytotron (UCT Botany Department). Temperature was set to 30/20°C (light/dark) and lights to: 9hr dark, 1hr step up, 13hr full intensity, 1 hr step down. Mean photosynthetic photon flux density (PPFD) in the phytotron was measured 2cm above pot surface using a Skye light meter (see **Table 2.2**). This was slightly lower for *Digitaria* as a lower stand was used. Gradients in PPFD (and temperature) existed between pot positions in the phytotron. Humidity could not be controlled but was measured regularly inside the phytotron. It rose as high as 90% during the dark period and dropped to approximately 50-60% during the light period.

Plastic rectangular pots (45cm x 30cm x 15cm) each holding approximately 23 litres of solution were used for all experiments. For *Digitaria* there were three plants per pot, for the other three species there 2 plants per pot except for the lowest treatment level (3 plants). For the first two experiments (*Digitaria* and *Urochloa*) black polyethylene plastic sheets were used to hold the plants approximately 1cm above the solution (**Fig. 2.4**). The plastic was cut every day or two to allow for lateral expansion of crowns and to allow roots emerging from stolon nodes to

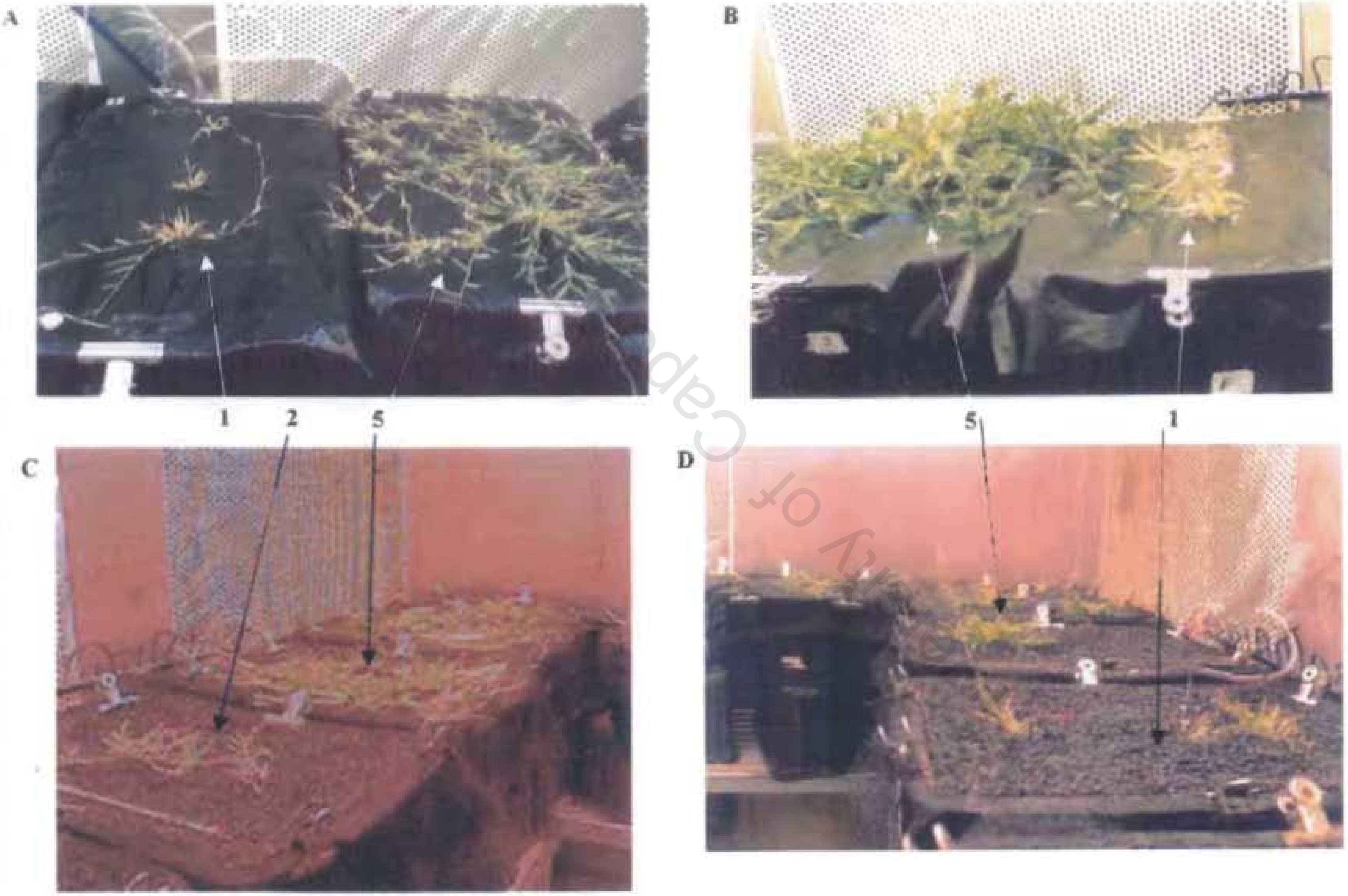


Figure 2.4. The solution culture experiment conducted in two phytotrons, University of Cape Town, showing **A.** *Digitaria longiflora*, **B.** *Urochloa mossambicensis*, **C.** *Dactyloctenium australe*, and **D.** *Sporobolus nitens*, shortly before the first harvests, after three to four weeks of growth. Plants were supported on either thick plastic (**A** and **B**) or in a bed of small plastic beads (**C** and **D**). Nitrogen was supplied as NH_4NO_3 at a range of concentrations - arrows show highest and lower treatment levels (see **Table 2.2**).

penetrate to the solution below. This method did not prove satisfactory, as many emerging roots died before reaching the solution, either as a result of the plastic not being cut in time, or the distance between the base of a node and the nutrient solution surface. In addition the temperature on the surface of the plastic rose as high as 52°C by the end of the photoperiod. Therefore, for the following two experiments (*Dactyloctenium* and *Sporobolus*) plants were grown in a bed of plastic beads (2-3 cm deep) held over a covering of shade cloth, in a method similar to that described by Asher and Edwards (1983). Due to sagging of the shade cloth, the lower part of the bead layer sat in the solution and the upper part was both humid and cooler than the plastic sheeting (maximum temperature recorded on the surface was 46° C).

After 3 (*Digitaria*), 4 (*Urochloa*), 5 (*Dactyloctenium*), or 9 (*Sporobolus*) days of growth, plants which were growing well were rinsed in DI water, stripped of dead leaves and roots and weighed, after blotting off excess water with tissue paper. This marked the beginning of each experiment. At the same time an additional 10-12 clones were weighed and dried to determine their moisture content. This value was then used to estimate the dry weight of clones used in the experiment. A N-free Long Ashton solution was used to supply nutrients, and this was renewed weekly. For the first week of the experiment, a half strength solution was used. Thereafter a full-strength solution was used. This contained the following concentrations of macronutrients (mM): 4 K^+, 4 Ca^{2+}, 3.5 SO_4^{2-}, 2.1 Na^+, 1.5 Mg^{2+} and 1.3 PO_4^{3-}; and micronutrients (:M): 8000 Cl^+; 140 BO_3^{3-}, 21 Mn^{2+}, 2.3 Zn^{2+}, 0.96 Cu^{2+}, 0.31 MoO_4^{2-} and 97 Fe^{2+} (as EDTA).

N was supplied as NH_4NO_3 (see **Table 2.2** for concentrations used for each level). For the highest level (40mM), plants were initially supplied with only 1mM N. This was raised to 40mM over the first 4 to 5 days of the experiment to allow for acclimation. In order to mitigate the effects the gradient in light intensity, PPFD was mapped using the average of 3 measurements at each pot position. Pots were then assigned a position so as minimize differences in average PPFD received by the two to three pots of each treatment level. In addition, when nutrient solutions were renewed, pots were shuffled to a different position relevant to their level. Pots were topped up with DI water every one or two days. pH and temperature of the solutions were monitored every 1-3 days (using a WTW ph 330 meter). pH fell between renewals for all species, and most rapidly at highest N supply. For the first experiment (*Digitaria*), pH was adjusted to up to 6.7 using 1M NaOH. However, this was found to cause precipitation when the acid was added ($CaPO_4$ is known to precipitate out of Long Ashton solution above a pH of 7 - Hewitt 1966). For the following three experiments, pH was allowed to fall to 5.8 and only adjusted up to 6.2. Minimum pH recorded between adjustments is shown in **Table 2.2** (pH did not fall below 6 for the lower three levels of the N gradient in any experiment). All plants were clipped to a height of 2cm every 2 weeks.

Table 2.2. Summary of the four solution culture experiments designed to test the effect of N supply rate on lawn grass growth. Dates, range in growth period, N concentrations supplied, and solution pH and temperature recorded and ranges in PPFD recorded prior to experiments. NR = not recorded.

Species	**Dates**	**Growth period (days)**	**N supply (mM)**							**pH range**	**Max. solution temp (°C)**	**PPFD min. (mean) max. (:mol m^{-2} s^{-1})**
			1	**2**	**3**	**4**	**5**	**6**	**7**			
Digitaria	Feb - Mar, 2001	25 – 44	0.009	0.040	0.16	0.63	2.4	10	40	6.3 - 6.8	NR	810 (925) 1080
Urochloa	May - Jul, 2001	18 – 46	0.016	0.052	0.21	1.0	40			5.2 - 6.8	32.7-39.1	1010 (1140) 1270
Dactyloctenium	Nov, 2001 - Jan, 2002	14 – 46	0.009	0.052	0.21	1.0	40			5.2 - 6.8	29.0 – 33.0	980 (1115) 1245
Sporobolus	Jan - Mar, 2002	22 – 49	0.009	0.052	0.21	1.0	40			5.1 - 6.8	29.0 – 33.0	NR

Growth measurements

For *Urochloa*, *Sporobolus* and *Dactyloctenium* leaf lengths were measured every 1, 2 or 3 days. Measurements (in mm) were from the point of emergence (juvenile leaves) or the ligule (adult leaves) up to the tip (or to the end of live tissue, if the tip was necrotic). For *Dactyloctenium* and *Sporobolus*, the number of nodal roots and shoots were counted at regular intervals. This became less frequent as the plants grew larger due to the difficulty of counting all the roots without removing plants from their solution. For *Urochloa*, *Dactyloctenium* and *Sporobolus*, root length was measured repeatedly (to the nearest half cm) for between one and five nodal roots per plant. This was only done for plants that had emerging roots at an accessible location (i.e. the near side of a pot). Roots emerging from stolon nodes were used for *Urochloa* and *Dactyloctenium*, and from the crown / rhizome for *Sporobolus*. The use of plastic beads made root measurements far more difficult for *Dactyloctenium* and *Sporobolus*, resulting in a far smaller sample. Numbers and dates of inflorescence production were recorded.

Harvest

It was hoped to harvest all plants at equal an size, to avoid the problem of ontogenetic drift (Coleman *et al.* 1994). However, due to time constraints and the slow growth of plants on the lowest N supply levels, plants on these levels were harvested at a far smaller size than those at higher N supply. Plants were allowed time to recover after a clip, and only harvested once the leaf area of the canopy had recovered to pre-clip proportions (judged visually). Harvested plants were separated into root, crown, culm, leaf sheath, leaf blade and standing dead (including detached dead leaves, attached dead leaves and necrotic leaf tips).

A subsample of about between 5 and 10 nodal roots, depending on the size of the plant, were selectively chosen to represent the full length range of nodal roots with laterals. The length of these roots was measured, their laterals root scrapped off by hand, and the axis and laterals weighed separately for each root.

Leaf blade and sheath area were measured separately using a CS51 scanner (*Digitaria* and *Urochloa*) or a LiCor 3100 area meter (LiCor, Inc.; *Dactyloctenium* and *Sporobolus*). For *Digitaria*, either all leaves on an individual (smaller plants) or a subsample of approximately a third of the total leaf area was measured. For the three other species, the wavy leaf margins made measuring all leaves together very difficult. Therefore, blades and sheaths were measured individually, for a small subsample, and weighed individually.

For the two strongly stoloniferous species, *Digitaria* and *Dactyloctenium*, the length of stolons was measured and the number of nodes, rooted nodes, nodal roots and stolon branches counted (this was only done for a subsample of the larger *Digitaria* plants). For *Dactyloctenium* only, mean branch angle was calculated by measuring the axil angle of 4 – 6 secondary stolons down the length of the longest primary stolon. All plant material was dried at 70°C to constant weight (2 to 3 days).

Response variables

Calculation

Without the advantage of repeated harvests, relative growth rate (**RGR**) can only be calculated as the mean RGR:

$$RGR = (\ln W_2 / W_1)/\Delta t$$

where W_2 is the dry weight at harvest, and W_1 is the initial dry weight, and Δt is the duration of growth. This model assumes that growth occurred at a constant exponential rate over the duration of the experiment. During the *Dactyloctenium* experiment, an electrical fault caused the lights to fail for two consecutive days. While some plants had already been harvested at this time, the remaining ones all became severely chloritic. All except those receiving 40mM N survived and recovered. Normal green colour as well as prior rates of shoot production only appeared to resume after a number of days after this incident. The number of days it took for the plants to regain previous form was subtracted from the total duration of growth when calculating RGR

For *Sporobolus*, **shoot number**, but not root number, at harvest proved to be a good predictor of dry weight (**Fig. 2.5**). Therefore, in order to test the assumption that growth at a constant exponential rate over the entire growth period, shoot number was ln-transformed and plotted against time. In addition, in order to test for any effects solution depletion and duration of experiment on growth rates, **leaf blade elongation** rates were calculated as daily growth rates: the increase in length between two consecutive daily measurements. This was done for *Urochloa* and *Sporobolus* only. Plots of blade length against time revealed that blade elongation was curvilinear, with an initial linear component for young leaves, and a rapid curve to a maximum as leaves matured. For each blade measured, plots were inspected visually and only the linear portion of growth included for calculations of daily growth. Daily growth rates were only calculated for the day prior to the day of solution renewal, and the day immediately after renewal.

Statistical analysis

Statistical analyses were done using STATISTA 6.0 (StatSoft 2001). RGR showed a curvilinear response to the gradient of N supply levels. Data for three of the species showed maxima at a low N supply which extended orders of magnitude, but fastest growing species did not reach a maximum. As a result commonly used asymptotic non-linear regressions, such as the monomolecular function and rectangular hyperbola (Causton and Dale 1990), did not provide accurate fits for all species over the entire N supply gradient. Instead a modified hyperbolic function was used:

$$RGR = N / (a + b.N + N^c)$$

where N is concentration of the N supplied. Curves were fitted using the Non-Linear Estimation module of STATISTICA 6.0, which uses the Levenberg-Marquardt algorithm to find the best least squares fit. 'Goodness of fit' of each fit was assessed on the basis of variance explained, the distribution of residuals and the significance and correlation of parameters. The fitted regressions were compared in a pairwise manner following Mead *et al.* (1993). For each comparison, a regression was fitted to combined data for two species, and a F ratio representing the increase in variance resulting from combining the data calculated as:

$$F = \frac{\underline{SS\ (combined) - SS\ (sum) / 2}}{MS\ (sum)}$$

where SS (combined) is the residual sum of squares from the regression of the combined data, SS (sum) is the sum of the residual sum of squares from the regressions for each species, and MS (sum) is the mean square calculated from the SS (sum). The significance of the F values obtained was determined from the F-distribution table in Mead *et al.* (1993). In addition to testing differences between response curves, maximum means were tested a General Linear Model (GLM) one-way ANOVA, using ln transformations where necessary.

Estimates of leaf blade daily elongation were tested using a Generalized Linear Model (GLZ) ANCOVA with species and solution renewal as main effects, and N supply and 'time of measure' (i.e. since the start of the growth period) as covariates. As daily growth showed a curvilinear response to N supply for both *Sporobolus* and *Urochloa*, a power link function was chosen (the 'power' parameter was set to 0.1, as determined using a non-linear power curve fit of leaf growth versus N supply). In addition, as variance of daily growth appeared to increase with its mean, the gamma (error) distribution option was chosen. An ANCOVA model testing homogeneity of slope revealed no significant interactions between either of the main effects and N supply or time of sampling. The ANCOVA was therefore rerun as a 'same slopes' model. The goodness of fit of this model was assessed on the basis of the deviation of the Pearson Chi-squared statistic, deviance statistic and log-likelihood values from 1 (StatSoft 2001).

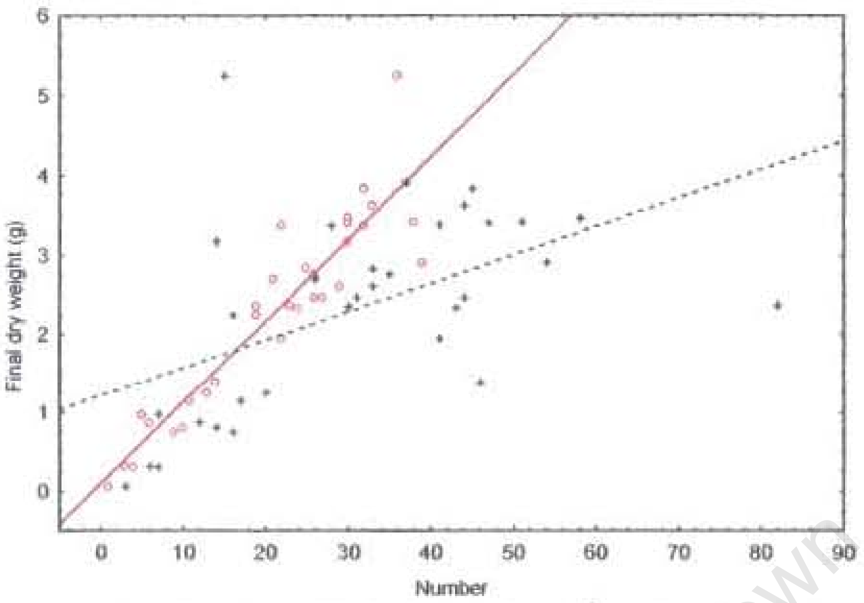


Figure 2.5. Final dry weight of *Sporobolus*, grown on five levels of NH_4NO_3 supply in solution culture, versus number of shoots: ↘ or roots: ↘. Linear least squares fits:
mass = 0.08 + 0.10*shoots; $r^2 = 0.86$; $p < 0.001$ and
mass = 1.22 + 0.036*roots; $r^2 = 0.27$; $p = 0.002$.

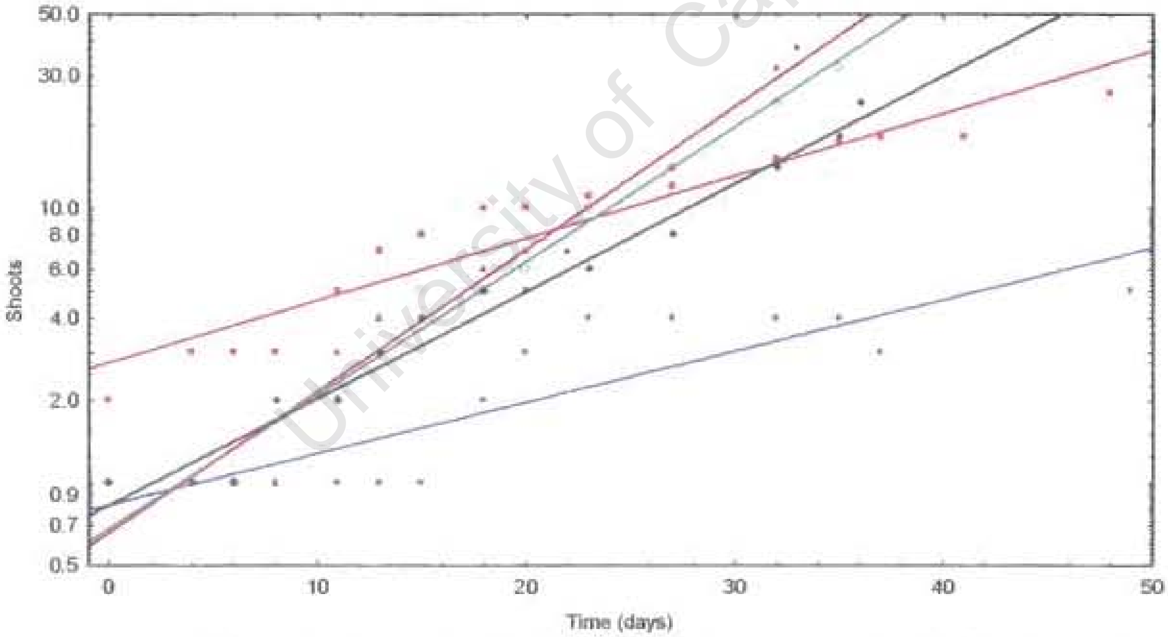


Figure 2.6. Increase in shoot number with time for *Sporobolus* grown on five levels of NH_4NO_3 supply in solution culture. N supply levels: **1**: ↘, **2**: ↘, **3**: ↘, **4**: ↘, **5**: ↘ (refer to **Table 2.2** for concentrations). Note the logarithmic y-axis. Log-linear regresssions were all significant below the 0.1% level.

RESULTS

Visual observations

All species exhibited deficiency or toxicity symptoms on the lower and highest N supply levels. On the lower levels, chlorosis was common, but least so for *Digitaria* and *Sporobolus* (see **Fig. 2.4**). On the 40mM N level, all species had very dark green leaves, with necrosis of the tips (even on young leaves). For *Urochloa* in particular, this necrosis was a dark brown colour, rather than the light brown colour of the necrotic leaf tips at the lower N levels. Also, many emerging leaves stuck together. Both of these symptoms may reflect Ca deficiency, although a dark green leaf colour is also a symptom of excess N supply (Jones 1998). *Dactyloctenium* plants developed a leaf fungal infection, with the area of blades infected ranging from 0-20%. This did not appear to affect the growth of the plants. A very small amount of salt secretion occurred on the leaf blades of *Digitaria* in the first few days of the experiment. *Sporobolus* produced large secretions on its blades (along leaf margins as well as the abaxial side of the midrib) for up to two weeks, across all treatments.

Growth rates

Estimation of RGR

For *Sporobolus*, the increase in shoot number over time was rather erratic, especially at lower N supply, presumably as a result of clipping. However, logarithmic transformation produced an overall linear relationship with time, regardless of N supply (**Fig. 2.6**) and the rate of shoot production did not appear to slow as the plants grew larger.

Daily leaf elongation rates for *Urochloa* and *Sporobolus* also indicate that growth did not slow as plants grew larger. The GLZ ANCOVA model indicated no significant effect of 'day of measure' (**Table 2.3**). However, 'solution renewal' was important and elongation was significantly slower when measured the day before renewal, as opposed to the day after renewal (**Fig. 2.7A**). This effect was in proportion to elongation rate when comparing between species,

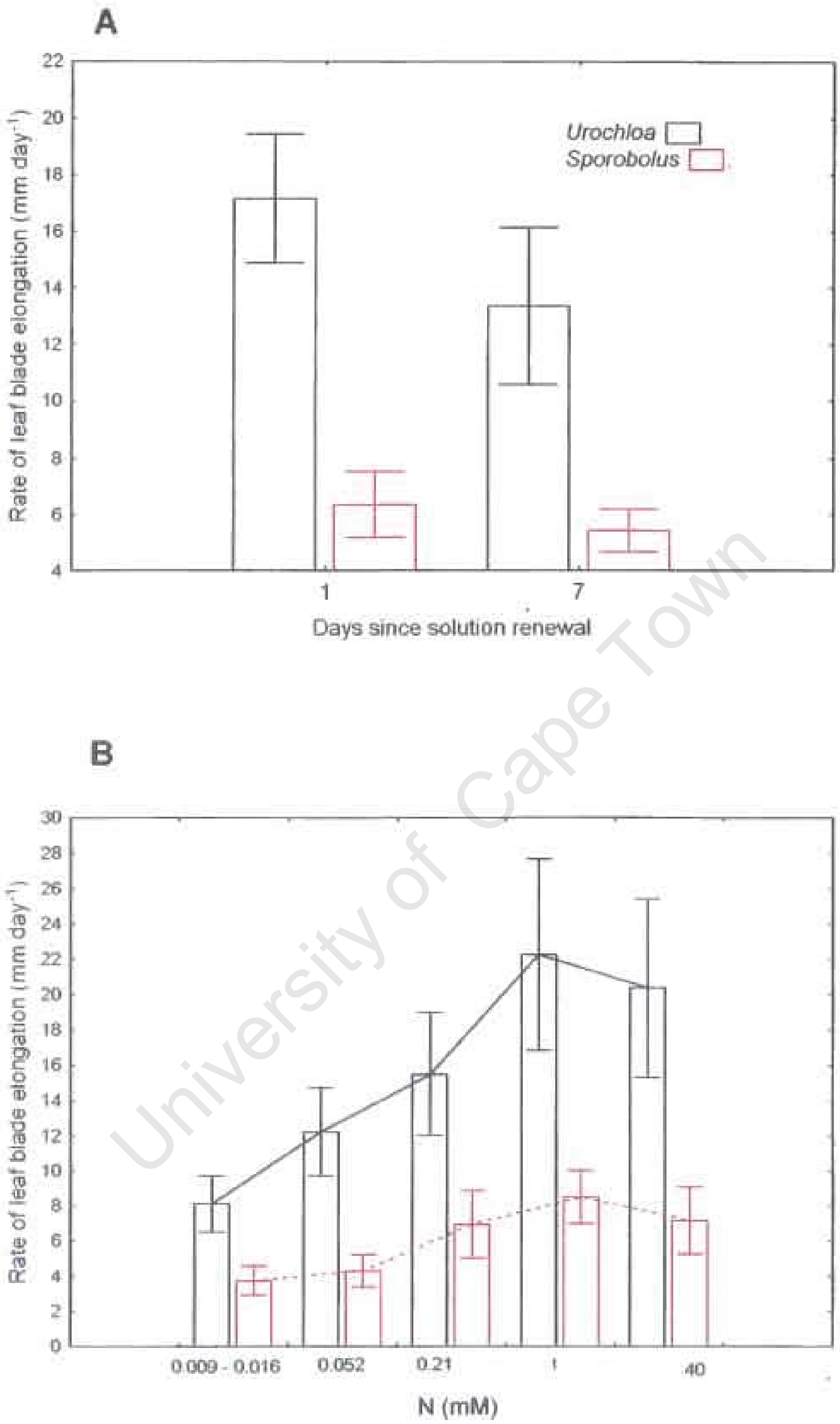


Figure 2.7. Daily leaf blade elongation for *Urochloa* and *Sporobolus* grown on at least five levels of NH_4NO_3 supply in solution culture. **A**. The effect of weekly solution renewal. **B**. The effect of the concentration of N supplied. Bars are means and whiskers show 95% confidence intervals. See **Table 2.3** for significance of the differences.

as there was no significant interaction between 'species' and 'solution renewal'. In terms of N supply, both species showed maximum daily growth at 1mm N supply (**Fig. 2.7B**). The decline of daily growth with N supply was again proportional when comparing species (no significant 'species x N supply' interaction the separate slopes ANCOVA – results not shown). Finally, comparing between species (and accounting for both the above effects) leaves of *Urochloa* elongated significantly faster than *Sporobolus*.

Table 2.3. Significance of the main effects (species and 'solution renewal') and covariates (N supply level and 'day of measure') of the GLZ ANCOVA model fitted to the daily leaf elongation data from *Urochloa* and *Sporobolus* grown on at least five levels of NH_4NO_3 supply in solution culture. The model used a power link function and a gamma error distribution. Goodness of fit statistics indicate that the model did provide an adequate fit.

Effect	**df**	**Wald statistic**	**p**
Intercept	1	5855.71	0.000
N supply	1	8.48	0.004
Day of measure	1	1.47	0.225
Species	1	81.06	0.000
Solution renewal	1	6.27	0.012
Species x Solution renewal	1	0.41	0.520
Goodness of fit	**df**	**Statistic**	**Stat/Df**
Deviance	339	226	0.67
Scaled Deviance	339	378	1.12
Pearson Chi	339	221	0.65
Scaled P. Chi	339	368	1.09
Log likelihood		-1140	

The biweekly clipping did not affect the four species in the same way. While clipping was clearly detrimental to growth at the lowest treatment level for all plants, this effect was not as clear as N supply increased, particularly for *Sporobolus* and *Dactyloctenium*. Faster growth at higher N supply did not result in significantly greater loss of shoot material per clip for these species (**Fig 2.8**). While there was a significant correlation between RGR and clipped mass for *Digitaria* and *Urochloa*, the effect was not particularly strong for the former (**Table 2.4**).

Table 2.4. Correlation oe-efficients for the mean dry weight of live material lost to biweekly clips and RGR for the four lawn grass species grown in solution culture on at least five levels of NH_4NO_3 supply.

Species	**n**	**r**	**p**
Urochloa	27	0.62	< 0.01
Dactyloctenium	29	0.24	> 0.05
Digitaria	38	0.49	< 0.01
Sporobolus	28	-0.11	> 0.05

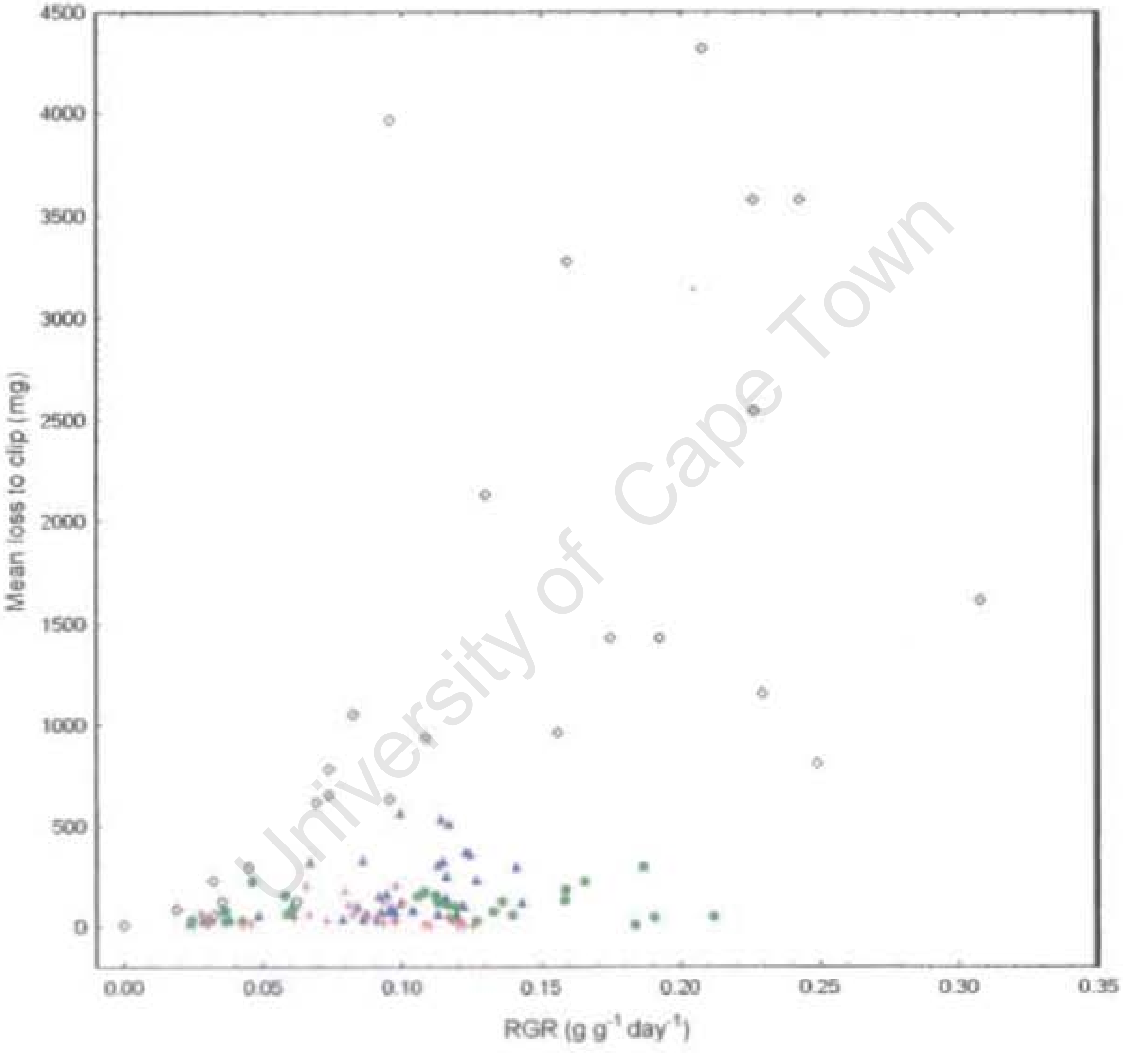


Figure 2.8. Mean biomass (dry weight) lost to biweekly clipping versus relative growth rate (RGR) for *Digitaria*: •, *Urochloa*: ○, *Dactyloctenium*: •, and *Sporobolus*: ·, grown on at least five levels of NH_4NO_3 supply in solution culture. Only *Urochloa* and *Digitaria* showed a significant increase in loss to clips with increasing RGR (see **Table 2.4**).

RGR

N supply affected the growth of all four lawn grass species with severe reductions in RGR at the lowest N supply levels (**Fig. 2.9**), resulting in significant fits of the non-linear regressions to the RGR data (**Table 2.5**).

Table 2.5. Summary of the non-linear fit: **$RGR = N / (a + b.N + N^c)$**, where **N** is the concentration of NH_4NO_3, for the four species of lawn grasses grown in solution culture on at least five levels of N supply. All fits were significant below $p = 0.01$. Unless indicated all parameters were significant below $p = 0.001$ (NS: $p > 0.05$)

Species	**n**	**r^2**	**SS residual**	**a (SE)**	**b (SE)**	**c (SE)**
Urochloa	27	0.91	0.016	0.29^{NS} (0.55)	3.81 (0.36)	0.37^{NS} (0.58)
Dactyloctenium	31	0.79	0.020	0.45 (0.11)	4.78 (0.42)	1.11 (0.11)
Digitaria	38	0.79	0.0029	0.19 (0.034)	7.51 (0.33)	1.24 (0.076)
Sporobolus	28	0.75	0.0060	0.18 (0.046)	8.40 (0.42)	1.09 (0.13)

Growth responses differed significantly between species, both in terms of the maximum RGR achieved at high N supply, and the rate at which RGR declined with declining N supply (**Table 2.6**). *Digitaria* and *Sporobolus* were an exception – both reached a similar maximum RGR, at a similar level of N supply, and showed only small differences in the way RGR declined in response to decreasing N supply. *Urochloa* showed the strongest growth response, with a high maximum RGR reached well above 1mM N (possibly even above 40mM N – **Fig. 2.9**). However, at intermediate N levels this species showed sharper decreases in RGR than the other three species, with its response curve falling below those of the others. *Urochloa* was not grown at the lowest N supply level but extrapolation of its response curve function to 0.009mM N suggests that this species had the lowest RGR at this level:

Urochloa (0.018 $g^{-1}g^{-1}day^{-1}$) < *Digitaria* (0.034) < *Dactyloctenium* (0.036) = *Sporobolus* (0.036).

This is the reverse of the ranking of the species in terms of maximum RGR. *Dactyloctenium* showed an intermediate response to that of *Urochloa* and *Digitaria* / *Sporobolus* at high and intermediate levels of N supply, but its growth decreased at a similar rate as for *Urochloa* at low N supply, resulting a similar RGR to *Digitaria* and *Sporobolus* at the lowest N supply level. For the two slowest growing species, *Digitaria* and *Sporobolus*, RGR decreased slightly at the highest N supply level.

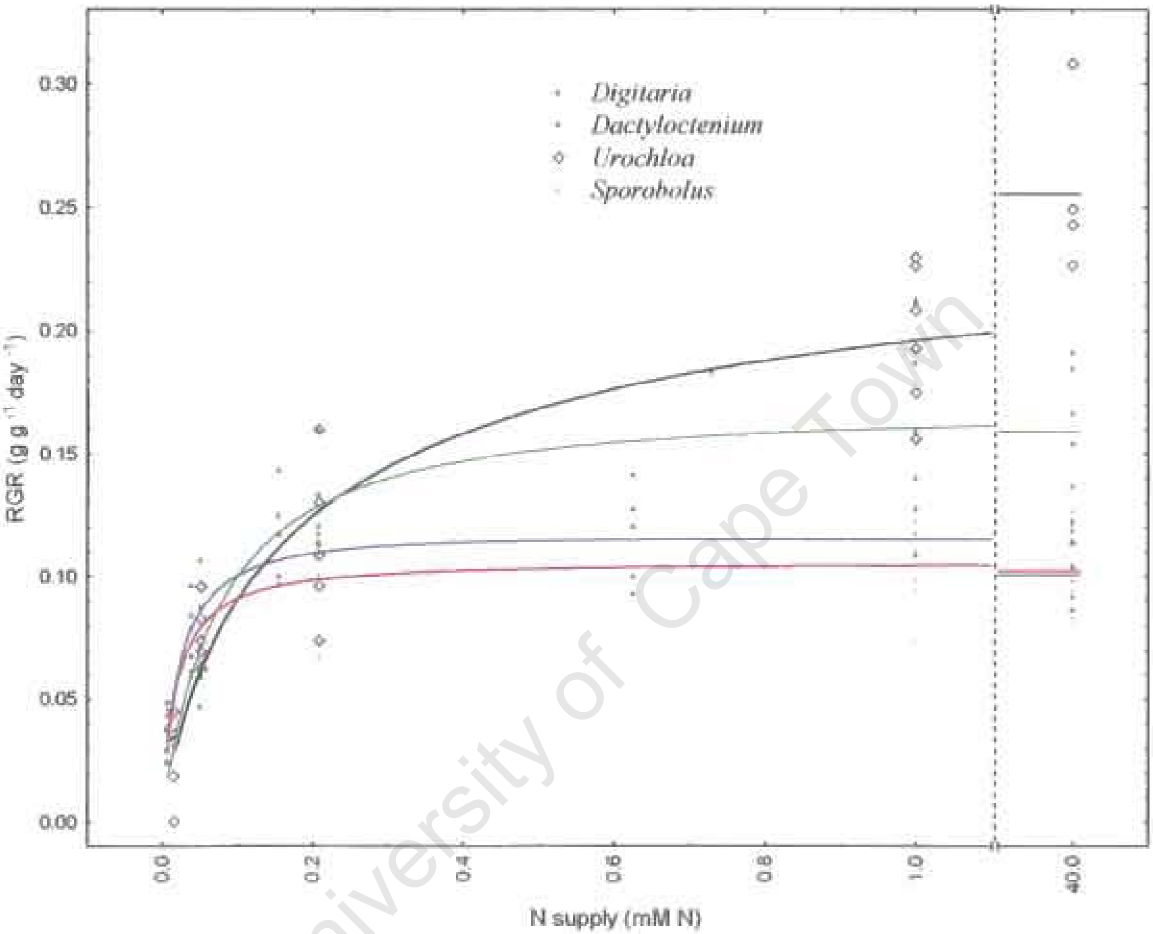


Figure 2.9. Relative growth rate (RGR) data for the four lawn grass species grown on at least five levels of NH_4NO_3 supply in solution culture. All curves fitted were significant (see **Table 2.5**). See **Table 2.6** for the significance of differences between the species.

Table 2.6. Pairwise comparisons of the RGR response curves and maximum RGR means for the four lawn grass species. F values and associated significance levels indicate differences between responses curves (NS: $p > 0.05$, *** $p < 0.001$). For maximum RGR data, means with the same subscript are not significantly different according to HSD post-hoc comparison for unequal sample sizes of a one-way ANOVA. N indicates the concentration levels of NH_4NO_3 supplied for which maximum values were taken. For *Urochloa* no RGR maximum was reached – values from the 40mM are assumed to be maximal.

Comparison F values				**Maximum RGR**		
	Dactyloctenium	*Digitaria*	*Sporobolus*	**N (mM)**	**Mean**	**n**
Urochloa	19.6^{***}	116^{***}	94.1^{***}	40	0.26^{a} (0.018)	4
Dactyloctenium	-	25.9^{***}	23.5^{***}	0.21 - 40	0.15^{b} (0.034)	19
Digitaria	-	-	1.11^{NS}	0.16 - 40	0.11^{c} (0.016)	26
Sporobolus	-	-	-	0.21 - 40	0.10^{c} (0.017)	18

DISCUSSION

Before discussing the significance of the observed differences in growth, the validity of the use of RGR is considered, both in terms of the accuracy of the RGR values estimated and in terms of how these values compare to other studies.

Validity of observed pattern

Estimation of RGR

Changes in shoot number and daily elongation rates of leaf blades suggest that for at least one of the species, constant exponential growth was prevented by both the biweekly clips and the intermittent solution renewal. However, since both shoot production and leaf elongation recovered rapidly after these 'disturbances' and, on average, shoot production appeared to remain exponential (**Fig 2.6**), the use of mean RGR to describe growth is justified. While the clipping treatment may have interfered with exponential growth, it may also have extended the duration of exponential growth by preventing self-shading, which often becomes a problem when estimating RGR (Poorter 1989).

In terms of estimating the response of RGR to N supply, the analysis of the leaf blade elongation data suggests that N was not supplied uniformly (in time), but rather as weekly pulses. If depletion between renewals was not in proportion to N supplied (as would be expected for plants growing at different exponential rates) or was greater for some species (as would be expected when RGR's differ) then this would have distorted the pattern of RGR response. Surprisingly though, reductions in leaf blade elongation appeared to occur more or less in proportion to N supply for both the fastest and slowest growing species (**Fig 2.7A**).

Another potential problem with the experimental design used is that it is unlikely that all other resources were supplied at a non-limiting rate across all N supply levels. While the effects of variation in light supply across the growth chamber may have been nullified by shuffling pot positions, maximum PPFD may have been too low. This is likely to a have affected plants at higher N supply more than at lower N supply, as light saturation is known to increase with plant N concentration (at least at the leaf level - Lambers *et al.* 1998). In addition, light saturation levels may have varied from species to species, thus distorting between-species comparisons. Wand *et al.* (2001) found that the light saturation of photosynthesis differed widely for eight South African C_4 grass species, although there was little increase in net assimilation rate above 1000:mol $m^{-2}s^{-1}$ of PAR. Attempts were made to measure light saturation at the leaf level, using an infrared gas analyser (Ciras 1, PP Systems Inc.) but the instrument malfunctioned before sufficient data could be collected. For the few leaves of *Urochloa* which were measured, light saturation was approximately 1400 :M m^{-2} s^{-2} for the 40mM N supply level. This suggests that RGR was underestimated at higher N supply (for *Urochloa*, at least). In addition the observed deficiency symptoms suggest that other nutrients, such as Ca, were beginning to limit growth.

Maximum RGR

The pattern of species with the highest maximum RGR showing the largest reductions in RGR in response to nutrient stress, as reported here, is a common result (Garnier 1998; Meziane and Shipley 1999). Garnier showed that there is a clear relationship between internal plant N concentration and RGR (as predicted analytically by Agren and Bosatta 1996). As Garnier proposes, it seems it is the inability of slow growing plants to achieve high levels of (internal) N concentration, even when N is provided at a high rate, which prevents them from achieving higher growth rates.

Response curves

The hyperbolic shape of the RGR responses seen here have been reported for other experiments with gradient designs (Ingestad and McDonald 1989 for light; Fichtner and Schulze 1992 for N; Poorter 1999 for light). With the exception of *Urochloa,* the level of N supply at which curves reached a maximum was surprisingly low. Robinson and Rorison (1985) reported maximum RGR at higher levels (0.21 to 4 mM N) in a flowing solution culture experiment for five grass species, although these were C_3 species. Furthermore, they found that the fastest growing species, *Holcus lanatus*, achieved maximum RGR at a lower supply of N than slower-growing species, the opposite of the pattern found here.

Ecological significance of the observed RGR responses

If one accepts that the RGR patterns observed were accurate, then the question arises whether these patterns constitute reliable evidence that the four species would differ in N response when grown under natural conditions. The limited evidence available in the literature regarding this question is discussed below.

Maximum RGR

The large divergence in maximum RGR between species suggests that the hypothesis that lawn grasses have a common, high requirement for N is false. Many authors have suggested that differences in maximum RGR reflect differences in habitat fertility, with the species from fertile habitats having a higher maximum RGR (Poorter and Remkes 1990; Fichtner and Schulze 1992; Grime 1994). However, while some of these studies provide evidence to support this, the pattern has not been tested extensively. Furthermore, Elberse and Berendse (1993) found a complete lack of correlation between 'habitat fertility' (as indexed by Ellenberg N numbers) and maximum RGR (and response of RGR to fertilization), using some of the same species used in the studies cited above. Also in contrast, Villar *et al* (1998) found a positive correlation between habitat mean annual rainfall and RGR for 20 grass species (presumably grown with a non-limiting supply of resources). This suggests that maximum RGR could reflect the availability of water in a habitat as much as it reflects nutrient availability. Therefore, while the results do suggest a low N demand for certain lawn grass species, this does not necessarily mean that N supply is low on grazing lawns where these species are common.

Response curves

Comparing RGR response curves between species the results suggest a trade-off, with the species with higher growth rates at high N supply having lower growth rates at low N supply. However, the large variance in the RGR data (within treatment levels), as well as the small number of N levels used at the lower end of the gradient, make this a tenuous conclusion at best. Such a trade-off is not generally considered to occur in response to nutrient supply (Lambers *et al.* 1998) and has not been found for water supply either (Fernandez and Reynolds 2000). A number of N addition experiments show that that regardless of RGR at high treatment levels, all species converge to similar low RGR at low N supply. This pattern has been found for individual plants under controlled conditions (Shipley and Keffy 1988, for emergent macrophytes; Fichtner and Schulze 1992, for annuals) and for populations in the field (Tilman and Wedin 1991, for 5 grass species). Furthermore, a lack of a trade-off was predicted by an analytical model of van der Werf *et al.* (1993) in which nitrogen productivity controlled RGR. Some studies even show that species with higher RGR at high N levels have higher RGR at low levels as well (Boot and Mensink 1990; Boot *et al.* 1990b, for 5 C3 grass species from the Netherlands), although in none of these studies was N supplied at a broad enough a gradient to rule out a reversal in species ranking at very low N supply.

In contrast to the above, Meziane and Shipley (1999) found a negative correlation between RGR at high and low nutrient supply (under conditions of high light supply) for 22 angiosperm species, which lends support to the pattern of a growth trade-off reported. Similarly, the results of Poorter (1999) indicate a trade-off in growth responses to light supply for forest trees.

To conclude this part, the four lawn grass species tested, changes in RGR in response to a uniform supply of inorganic N do not indicate a common, high demand for N. Instead, there is evidence for resource partitioning, with the ranking of species reversing at 'high' and 'low' rates of N supply.

PART 2

ALLOCATION PATTERNS

METHOD

Experimental design

Species used, growth conditions, growth measurements and harvest measurements were described in part 1 of this section.

Response variables

Organ allocation

Biomass allocations were calculated as 'mass fractions' which are preferable to ratios (Poorter and Nagel 2000). Each mass fraction is the organ's dry weight divided by total plant dry weight (for roots: **RMF**, leaf blades: **LMF_B**, leaf sheaths: **LMF_S**, culms: **CMF**, crowns / rhizomes: **CrMF**). In addition, the fraction of blade weight over total leaf weight (**BMF**) and the fraction of lateral root weight over total nodal root weight (**LatMF**) was calculated. The dry weight of the standing dead was used to calculate fraction of above-ground material (including crowns) which was dead (**DMF_{AG}**). This was not done for *Dactyloctenium* due to the excess standing dead which accumulated after the two day power failure.

Nodal root variables

The number of nodal roots per plant, together with final dry weight data were used to calculate the ratio of **'nodal roots / AG biomass'** and **'mean nodal root mass / AG mass'** for each plant.

The specific root length of nodal roots stripped of laterals (**SRL_N**) was calculated as 'nodal root length / nodal root weight'. Note that this is not the same as the conventional calculation of SRL, which involves measurements of the length and weight of sub samples of an entire root (or root system), including laterals. **Nodal root elongation** was assumed to be linear and calculated as the slope of length versus time. For *Urochloa* elongation curves often appeared to reach an asymptote, but for roots measured over long time, linear growth resumed (**Fig 2.10**). A similar pattern has been observed for other fibrous root systems (Lecompte *et al.* 2001). Only the steepest linear portion of a root growth curve was used for calculating elongation rates for *Urochloa*. Slope was determined by a least squares linear fit: durations of linear growth ranged from 3 - 20 days, the number of consecutive measurements ranged from 3 - 13, r^2 ranged from 0.94 - >0.99. For *Dactyloctenium* and *Sporobolus*, only 2 or 3 measurements, including one at harvest, were available. These points were checked for linearity graphically, and elongation rates calculated as the difference between final and initial length. When more than one root was measured per plant, mean values were used.

Ramet placement

For *Digitaria* and *Dactyloctenium*, **branching intensity** was calculated as the total number of stolons produced (including those from the original ramet and those which were produced as branches from stolon nodes) divided by the total number of nodes (the original ramet was counted as one node). **Mean internode length** was the total length of the stolons divided by the total number of nodes. Specific culm length (**SCL**) was calculated as the total length of all stolons divided by total stolon dry weight.

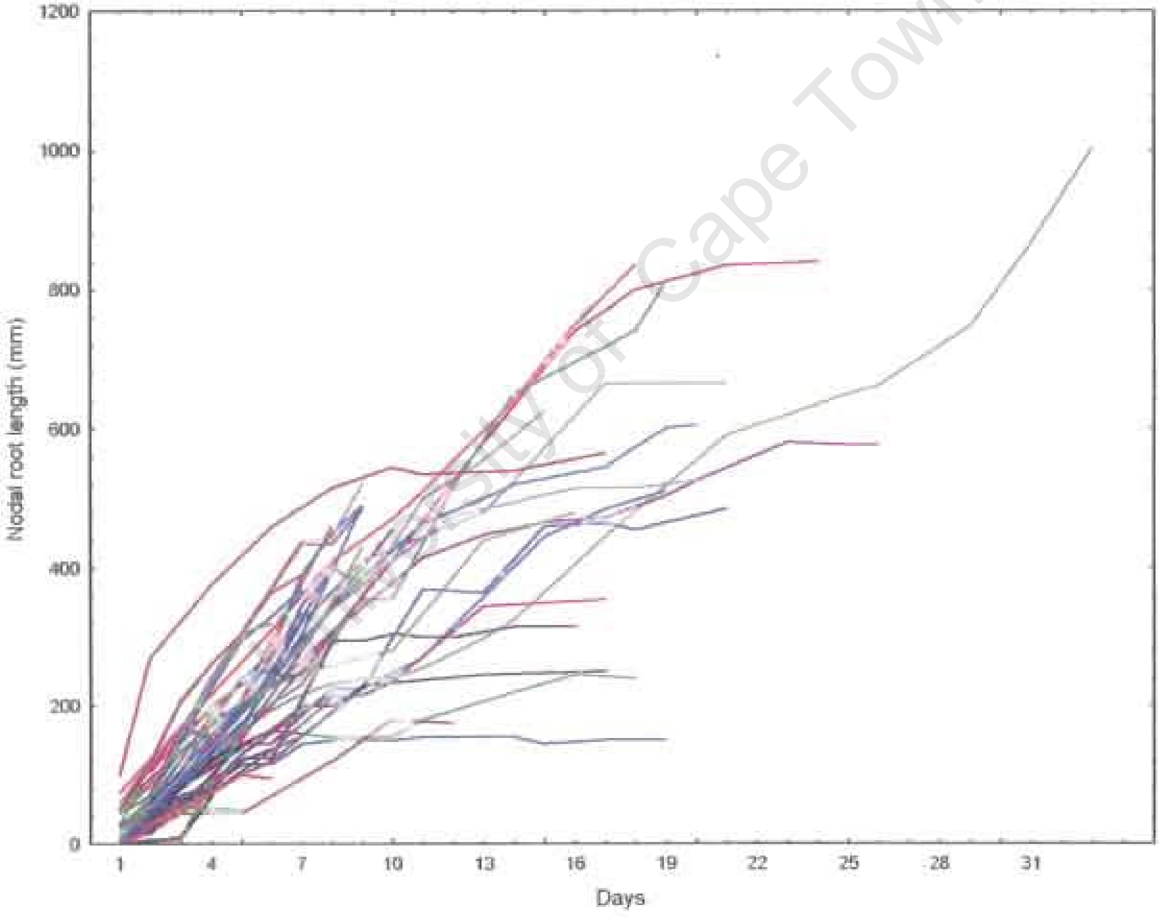


Figure 2.10. Line plots of root elongation for the majority of nodal roots measured for *Urochloa* plants grown on five levels of NH_4NO_3 supply in solution culture. Roots were measured approximately every two days.

Leaf area variables

For *Urochloa*, *Dactyloctenium* and *Sporobolus*, least squares linear regressions were fitted for each leaf subsample as:

$$\text{blade area} = \text{b.(blade mass)} + \text{c.}$$

Provided that the relationship between area and mass is isometric, i.e. linear and passes through the origin ($c = 0$), the slope (b) gives the specific leaf area of leaf blades (**SLA_B**). For many of the subsamples, the variance in blade area increased with blade mass. This was accounted for by using weighted values (1 / blade mass) in the regression analyses. For almost all subsamples, intercepts were not significantly different from zero, and linear fits were significant below the 1% confidence level (**Table 2.6**).

This suggests that there was no change in SLA_B as leaves grew (which has been observed for a range broad-leaf dicotyledonous species as well - Roderick and Cochrane 2002). Plants for which subsamples had significant intercepts and / or poor fits were excluded. Values of SLA_B were used to extrapolate the total leaf blade area per plant, which was used to calculate the ratio of leaf blade area to total plant dry weight (**LAR_B**).

Table 2.6. Regression coefficients from linear least squares regression of leaf area against leaf mass. See text for details.

Species	N	range of r^2
Urochloa	5 – 15	0.86 – 0.99
Dactyloctenium	8 – 15	0.83 – 0.99
Sporobolus	9 – 14	0.95 – 0.99

Statistical analysis

Organ allocation

Difference in organ level biomass allocation were analysed in the same manner as RGR in part 1 of this section. However, the extended hyperbola function used for the non-linear regression was modified slightly to account for differences in response shapes. While some of the parameters were not significant, and some fits showed a high correlation between parameters, these functions were maintained as they were the only functions which provided acceptable fits for all four species, for each variable. This allowed for pairwise tests for significant differences between the regression fitted for each species, as in section 1. The regression used, and the parameters estimated are given in **Table 2.7.**

Nodal root patterns

Both SRL_N and LatMF were affected by nodal root length. To test for both differences between species, and the effect of N supply, GLM ANCOVA models (separate ones for SRL_N and LatMF) were therefore used, with nodal root length and N supply as covariates ('species' was set as a random factor – as each species was tested in separate experiments, at different times). By using an ANCOVA model designed to test the homogeneity of slopes, differences between species (taking into account the effects of root length and N supply) could be tested as the significance of the 'species x N supply x root length' interaction. In all cases, the response variable and nodal root length were ln transformed, to satisfy the assumptions of linearity and homogeneity of variance. Residuals of the models were tested using normal probability plots and plots of raw residuals versus predicted values.

Table 2.7. Non-linear regressions fitted to the allocation variable data for the four lawn grass species grown in solution culture on at least five levels of NH_4NO_3 supply. Parameters and associated standard errors are shown. All fits were significant below $p = 0.01$. Unless indicated all parameters were significant below $p = 0.001$ (NS: $p > 0.05$, * $p > 0.01$). N is the concentration of NH_4NO_3 (see text for explanation of the allocation variable acronyms).

Species	n	r^2	SS	a (SE)	b (SE)	c (SE)
RMF = a.N + b.N^{-c}						
Urochloa	26	0.63	0.17	0.0004^{NS} (0.001)	0.27 (0.029)	0.15 (0.035)
Dactyloctenium	31	0.86	0.096	0.0021 (0.0007)	0.17 (0.015)	0.24 (0.023)
Digitaria	38	0.78	0.11	0.0020 (0.0006)	0.12 (0.013)	0.25 (0.029)
Sporobolus	25	0.91	0.043	0.0033 (0.0005)	0.11 (0.0011)	0.33 (0.026)
LAR_B = N / (a.N + b.N^{-c})						
Urochloa	22	0.64	6.30	0.17 (0.033)	0.092^{*} (0.041)	0.66 (0.11)
Dactyloctenium	27	0.71	69.1	0.11 (0.007)	0.002^{NS} (0.002)	-0.077^{NS} (0.29)
Digitaria	32	0.61	25.6	0.19 (0.014)	0.018^{NS} (0.015)	0.40^{NS} (0.20)
Sporobolus	25	0.69	27.8	0.13 (0.007)	0.009^{NS} (0.007)	0.36^{NS} (0.18)
LMF_B = N / (a.N + b.N^{-c})						
Urochloa	26	0.81	0.033	2.56 (0.41)	1.60 (0.57)	0.65 (0.10)
Dactyloctenium	31	0.61	0.073	3.30 (0.27)	0.39^{NS} (0.28)	0.47^{*} (0.17)
Digitaria	32	0.55	0.13	5.72 (0.42)	0.58^{NS} (0.47)	0.61 (0.17)
Sporobolus	29	0.79	0.053	2.10 (0.067)	0.040^{NS} (0.034)	0.19^{NS} (0.19)
SLA_B = N/(a + b.N + N^{-c})						
Urochloa	22	NS				
Dactyloctenium	27	0.71	372	-1.00 (0.0002)	0.029 (0.001)	-0.0001^{NS} (0.0001)
Digitaria	32	0.53	461	- 1.00 (0.0004)	0.034 (0.001)	0.0001^{NS} (0.0001)
Sporobolus	25	NS				
DMF_{AG} = a.N + b.N^{-c}						
Urochloa	26	0.75	0.11	-0.0005^{NS} (0.001)	0.16 (0.022)	0.22 (0.043)
Digitaria	38	0.67	0.089	0.0027 (0.0005)	0.026 (0.009)	0.48 (0.08)
Sporobolus	25	0.76	0.077	0.0042 (0.0007)	0.094 (0.016)	0.31 (0.042)

RESULTS

Visual observations

Leaf longevity was observed to be shorter at lower N supply. This appeared to result from leaves dying before reaching an adult size, and at the lowest N supply level most leaves died before the ligule had formed (but the few leaves which did survive to the adult stage often grew as large as leaves on the higher N levels).

Rhizomes barely developed in *Sporobolus* – for most plants the 'rhizome' resembled the crowns of the other three species, being more or less circular in shape and without scale leaves. However, in a few of the large plants a small amount of elongation had begun.

Urochloa showed a herringbone pattern of lateral root branching, with many short and mostly unbranched primary laterals. *Digitaria* was the opposite with long primary and secondary laterals, while *Dactyloctenium* was intermediate. *Sporobolus* showed the most variable root architecture: nodal roots from the 40mM level were similar to *Urochloa*, with short, thick laterals; those from the intermediate levels were similar to *Digitaria*; and those from the lowest N level again appeared to show a decline in lateral branching. Uneven proliferation of lateral roots down the nodal axis was also observed for *Sporobolus*.

Dactyloctenium was the only species not to produce flowers. Numbers of individuals that flowered and the N supply on which they were grown, is shown in **Table 2.8**. In some cases developing inflorescences were below a height of 2cm at the time of a clip and subsequently matured (to the stage of pollen production) unhindered. When developing inflorescences were cut, the lower residual portion continued to develop. For *Sporobolus*, some of the flowering culms that were clipped simply branched and produced a new inflorescence in the following inter-clip interval.

Table 2.8. Time (days from the start of the experiment) for inflorescence development for the three lawn grass species which flowered. Each number represents a different individual, * indicates individuals which re-flowered after losing their first inflorescence to a clip.

N supply (mM N)	*Digitaria*	*Urochloa*	*Sporobolus*
0.009			20
0.040	24, 26		
0.052			13, 13, 20*, 20*
0.16	26		
0.21		26, 27, 34	
0.63	26		
2.44	23, 25		
10	25		

Allocation to root

RMF

Adjustments in RMF were the inverse of changes in RGR (**Fig 2.11A**) and significant negative correlations were found between RGR and RMF within each species (**Table 2.9**). *Sporobolus* and *Digitaria* again showed similar changes, but the latter reached slightly lower minimum RMF at intermediate levels of N supply, and former had higher maximum RMF at low N supply, resulting in significantly differences in the RMF response curves (**Table 2.10**). *Urochloa* had the highest RMF at high N supply, but increased allocation to root the least in response to decreasing N supply, resulting in a similar maximum RMF as for the two slower growing species. *Dactyloctenium* was again intermediate between these species, but increased RMF rapidly in response to low N supply. Extrapolation of the response curve function for *Urochloa* gave the following ranking of the species at 0.009mM N:

Dactyloctenium (0.61 $g^{-1}g^{-1}$) > *Sporobolus* (0.56) > *Urochloa* (0.55) > *Digitaria* (0.50).

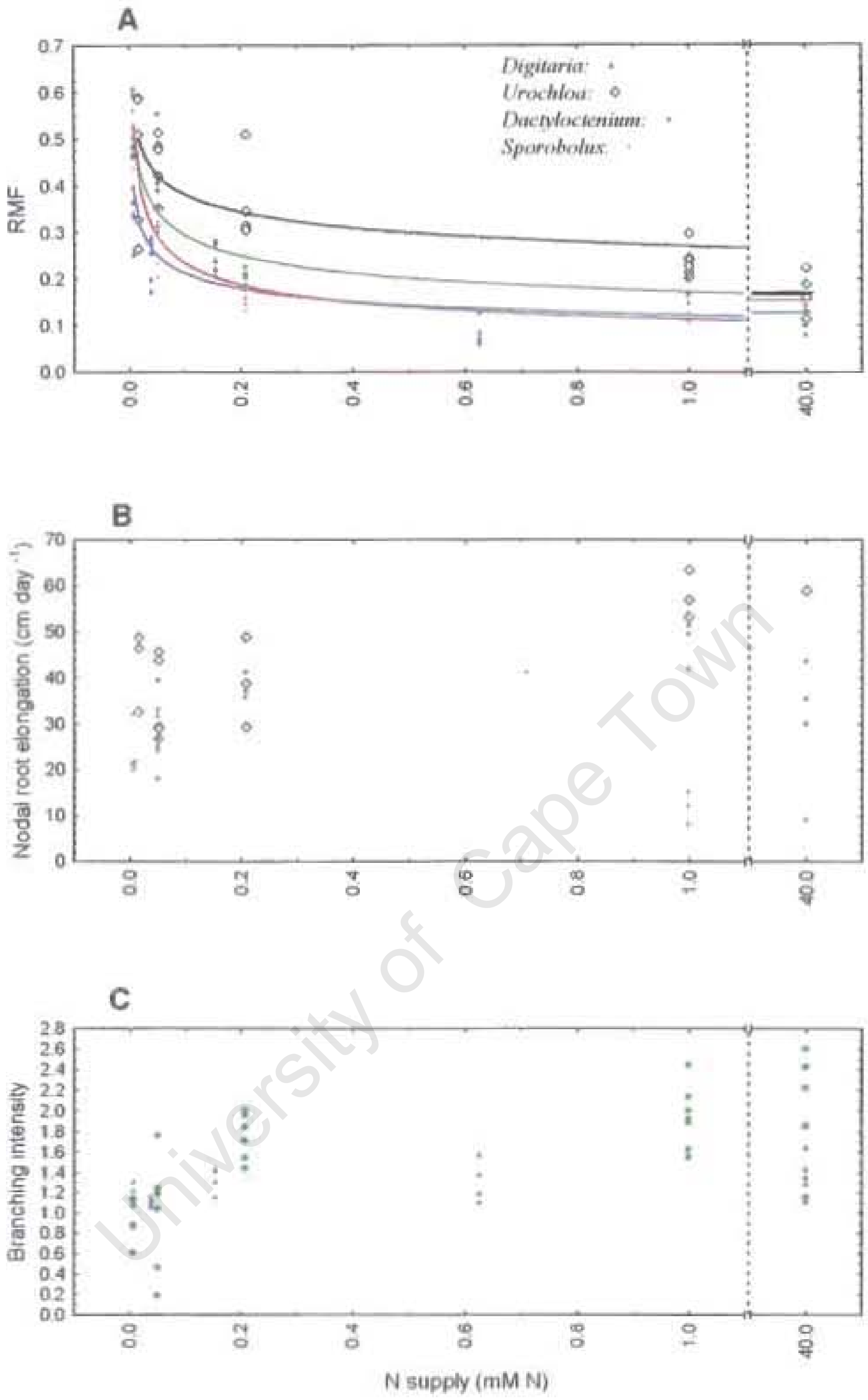


Figure 2.11. Biomass allocation and morphological growth responses for the four lawn grass species grown on at least five levels of NH_4NO_3 supply in solution culture. **A.** Root mass fraction (RMF), **B.** rate of nodal root elongation, **C.** stolon branching intensity, **D.** mean stolon internode length, **E.** leaf (blade) area ratio (LAR_B), **F.** leaf (blade) mass fraction (LMF_B), **G.** specific leaf (blade) area (SLA_B), **H.** the fraction of leaves comprised of blades (BMF), and **I.** the fraction of total above-ground mass comprosied of dead material (DMG_{AG}). All curves fitted were significant (see **Table 2.7**) and significantly different from each other (see **Table 2.10**).

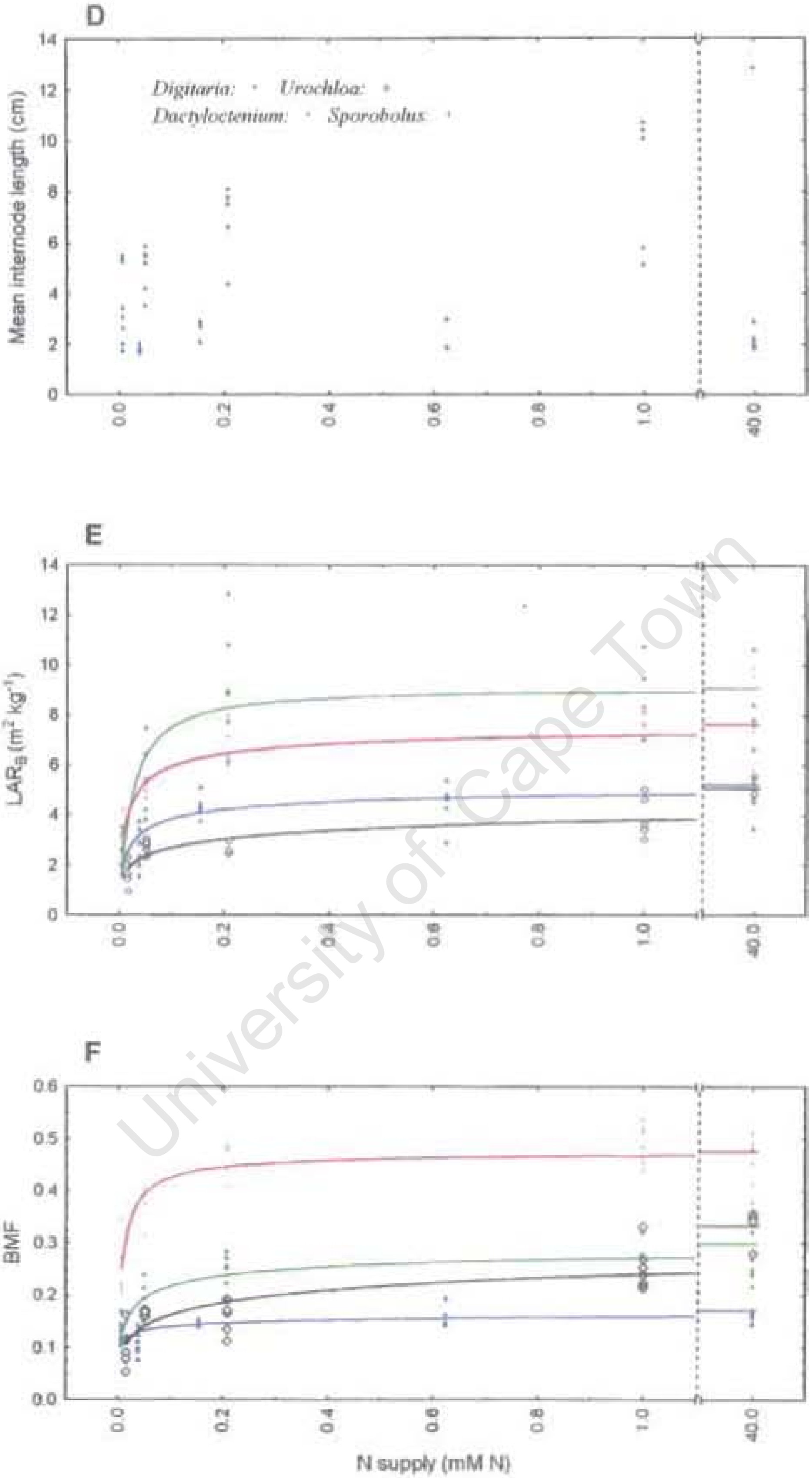


Figure 2.11. Cont'd

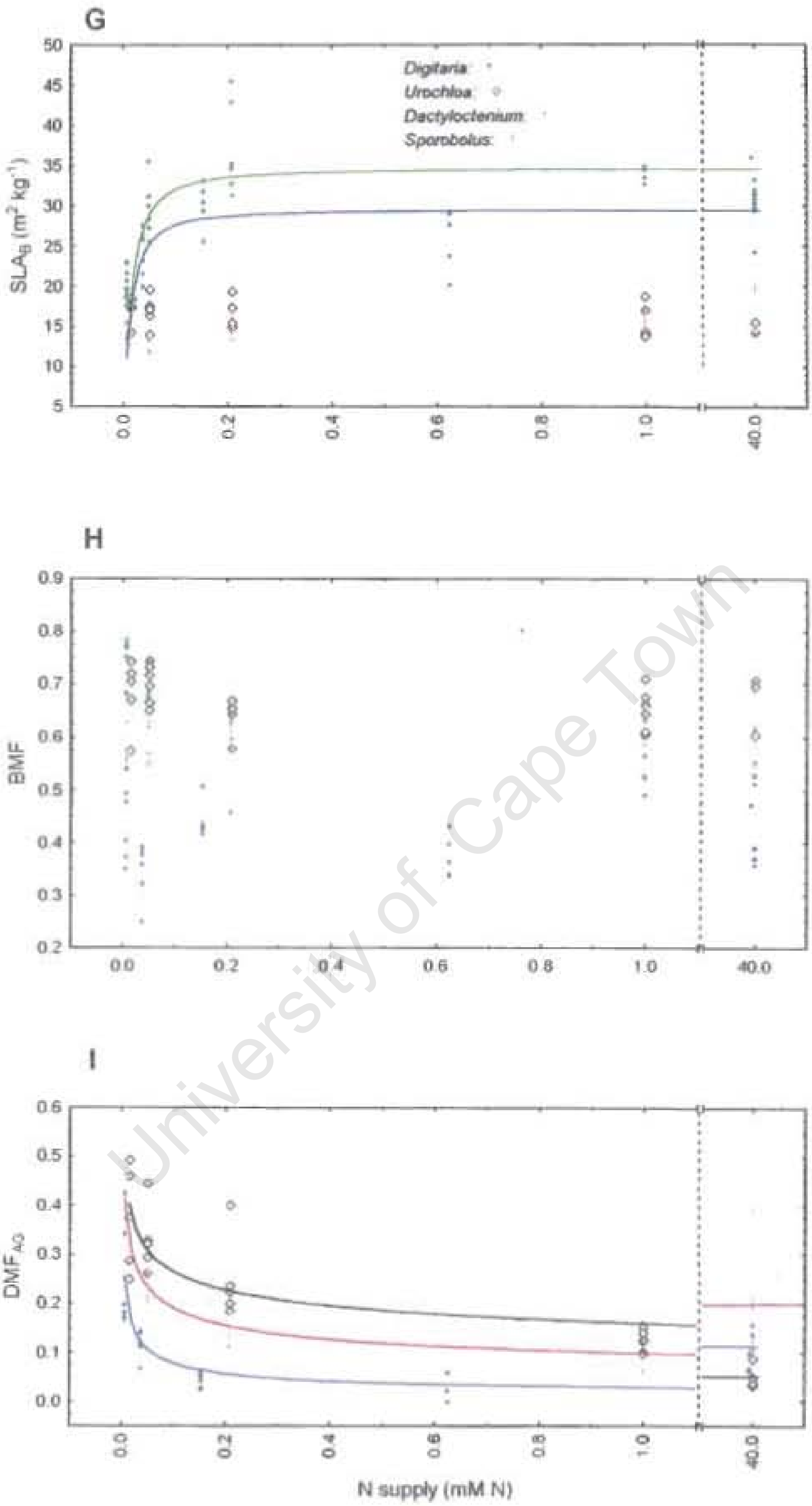


Figure 2.11. Continued

Table 2.9. Correlations between relative growth rate (RGR) and allocation variables, and between leaf area variables and biomass allocation variables (see **Fig. 2.11** for explanation of acronyms). Within species correlations are Pearsons product moment co-efficients, between species are Spearman'a rank co-efficients. Significance levels: NS $p > 0.05$, ** $p < 0.01$, *** $p < 0.001$

Within species		n	r	p
RGR vs:				
RMF	*Urochloa*	26	-0.81	***
	Dactyloctenium	31	-0.88	***
	Digitaria	38	-0.68	***
	Sporobolus	25	-0.78	***
LAR$_B$	*Urochloa*	22	0.82	***
	Dactyloctenium	27	0.70	***
	Digitaria	32	0.72	***
	Sporobolus	25	0.85	***
LMF$_B$	*Urochloa*	26	0.88	***
	Dactyloctenium	31	0.61	***
	Digitaria	32	0.70	***
	Sporobolus	27	0.88	***
SLA$_B$	*Urochloa*	22	-0.40	NS
	Dactyloctenium	27	0.63	***
	Digitaria	32	0.68	***
	Sporobolus	26	0.52	**
DMF$_{AG}$	*Urochloa*	26	-0.81	***
	Dactyloctenium		n.d.	
	Digitaria	38	-0.74	***
	Sporobolus	25	-0.87	***
LAR$_B$ vs:				
LMF$_B$	*Urochloa*	22	0.96	***
	Dactyloctenium	27	0.95	***
	Digitaria	32	0.91	***
	Sporobolus	25	0.91	***
SLA$_B$	*Urochloa*	22	-0.27	NS
	Dactyloctenium	27	0.91	***
	Digitaria	32	0.91	***
	Sporobolus	25	0.79	***

Between species (at optimum N)	n	r	p
RGR vs:			
RMF	4	0.80	NS
LAR$_B$	4	0.00	NS
LMF$_B$	4	-0.20	NS
SLA$_B$	4	-0.20	NS
DMF$_{AG}$	3	-0.50	NS
LAR$_B$ vs:			
LMF$_B$	4	0.40	NS
SLA$_B$	4	0.40	NS
SLA$_B$ vs:			
DMF$_{AG}$	3	1	NS

Table 2.10. Pairwise comparisons of the response curves and mean maxima / minima for the various allocation variables estimated for the four lawn grass species grown in solution culture. F values and associated significance levels indicate differences between responses curves (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; NT = not tested). Maxima / minima means with the same subscript are not significantly different according to HSD post-hoc tests for unequal sample sizes of one-way ANOVA's. N indicates the concentration level of NH_4NO_3 supplied for which maxima / minima values were taken. For *Urochloa* no maxima and minima were reached – values from the 40mM treatment level are assumed to be maximal / minimal. ND = no data. (see **Fig. 2.11** for explanation of the allocation variable acronyms).

Variable	**Comparison F values**			**Minima / maxima**		
				N (mM)	**Mean**	**n**
RMF	*Dactyloctenium*	*Digitaria*	*Sporobolus*		Minimum	
Urochloa	6.91^{**}	30.6^{***}	18.9^{***}	40	0.17^{a} (0.046)	4
Dactyloctenium	-	17.5^{***}	5.91^{**}	1.0 – 40	0.16^{a} (0.028)	13
Digitaria	-	-	4.91^{*}	0.63 – 10	0.096^{b} (0.030)	16
Sporobolus	-	-	-	1.0	0.12^{ab} (0.018)	5
LAR_B	*Dactyloctenium*	*Digitaria*	*Sporobolus*		Minimum	
Urochloa	54.1^{**}	526^{***}	59.8^{***}	40	5.21^{ab} (0.458)	2
Dactyloctenium	-	43.7^{***}	4.65^{*}	0.21 - 40	8.79^{b} (1.83)	15
Digitaria	-	-	20.2^{***}	0.24 – 40	4.97^{a} (1.06)	7
Sporobolus	-	-	-	1.0 – 40	7.70^{b} (1.36)	11
LMF_B	*Dactyloctenium*	*Digitaria*	*Sporobolus*		Maximum	
Urochloa	6.26^{**}	52.9^{***}	185^{***}	40	0.332^{b} (0.0353)	4
Dactyloctenium	-	44.1^{***}	109^{***}	1 – 40	0.284^{b} (0.0689)	13
Digitaria	-	-	246^{***}	0.63 - 2.4	0.175^{c} (0.0208)	8
Sporobolus	-	-	-	1.0	0.491^{a} (0.0380)	6
SLA_B	*Dactyloctenium*	*Digitaria*	*Sporobolus*		Maximum	
Urochloa	NT	NT	NT	all	16.5^{a} (1.86)	22
Dactyloctenium	-	9.96^{***}	NT	0.21 - 1.0	35.8^{c} (4.63)	10
Digitaria	-	-	NT	0.16 - 40	29.1^{b} (4.22)	20
Sporobolus	-	-	-	0.21 - 40	15.8^{a} (1.84)	16
DMF_{AG}	*Dactyloctenium*	*Digitaria*	*Sporobolus*		Minimum	
Urochloa	NT	54.0^{***}	11.2^{***}	40	0.050^{a} (0.025)	4
Dactyloctenium	-	NT	NT	ND		
Digitaria	-	-	19.1^{***}	1	0.12^{b} (0.042)	5
Sporobolus	-	-	-	0.16 - 2.4	0.041^{a} (0.020)	15

Optimum RMF

For *Digitaria* and *Sporobolus* optimum N supply was not simply the range of N supplies at which maximum RGR was achieved – above 1mM N these species had to increase allocation to roots to maintain maximum RGR. The term **optimum** is therefore used hereafter to denote the level(s) of N supply at which all response variables (including biomass allocation variables) were at their maximum / minimum. Optimum RGR and optimum RMF showed a positive correlation between species, but this was not significant due to a rank-reversal of *Digitaria* and *Sporobolus* (**Table 2.9**). As a result, total adjustments in RMF (the range from optimum RMF to RMF at 0.009mM) did not match changes in RGR either, with ranking:

Dactyloctenium (0.45 $g^{-1}g^{-1}$) > *Sporobolus* (0.44) > *Digitaria* (0.41) > *Urochloa* (0.38).

Components of RMF

Nodal root number and mass

For all species, relationships between both 'nodal roots / AG mass' and RMF, and 'mean nodal root mass / AG mass' and RMF were non-linear (**Fig. 2.12**). For intermediate values of RMF, 'nodal roots / AG mass' appeared to show a reasonably good (linear) predictor of RMF for *Digitaria* and *Urochloa*, while 'mean nodal root mass / AG mass' appeared to be a better predictor for *Sporobolus* and *Dactyloctenium*. However, for the lowest and highest values of these component variables, variance in RMF was large.

Nodal root elongation rate

Increases in 'mean nodal root mass / AG mass' can be deconstructed into two component parts, as the mass of each nodal root is the product of the growth of the nodal axis and its lateral branches. The data collected for **nodal root elongation** does not include a relative measure of AG growth, and cannot be used to test for differences between species. Nevertheless, a striking pattern was observed from the small sample of data collected (**Fig. 2.11B**). At lower N supply *Urochloa* and *Dactyloctenium* showed a decline in elongation rates, while *Sporobolus* showed a slight increase. Maximum rates of nodal root elongation appeared to reflected RGR – at high N supply, the fastest growing species had the highest rates of elongation, while at low N supply, all species converged to similar values. The highest values for

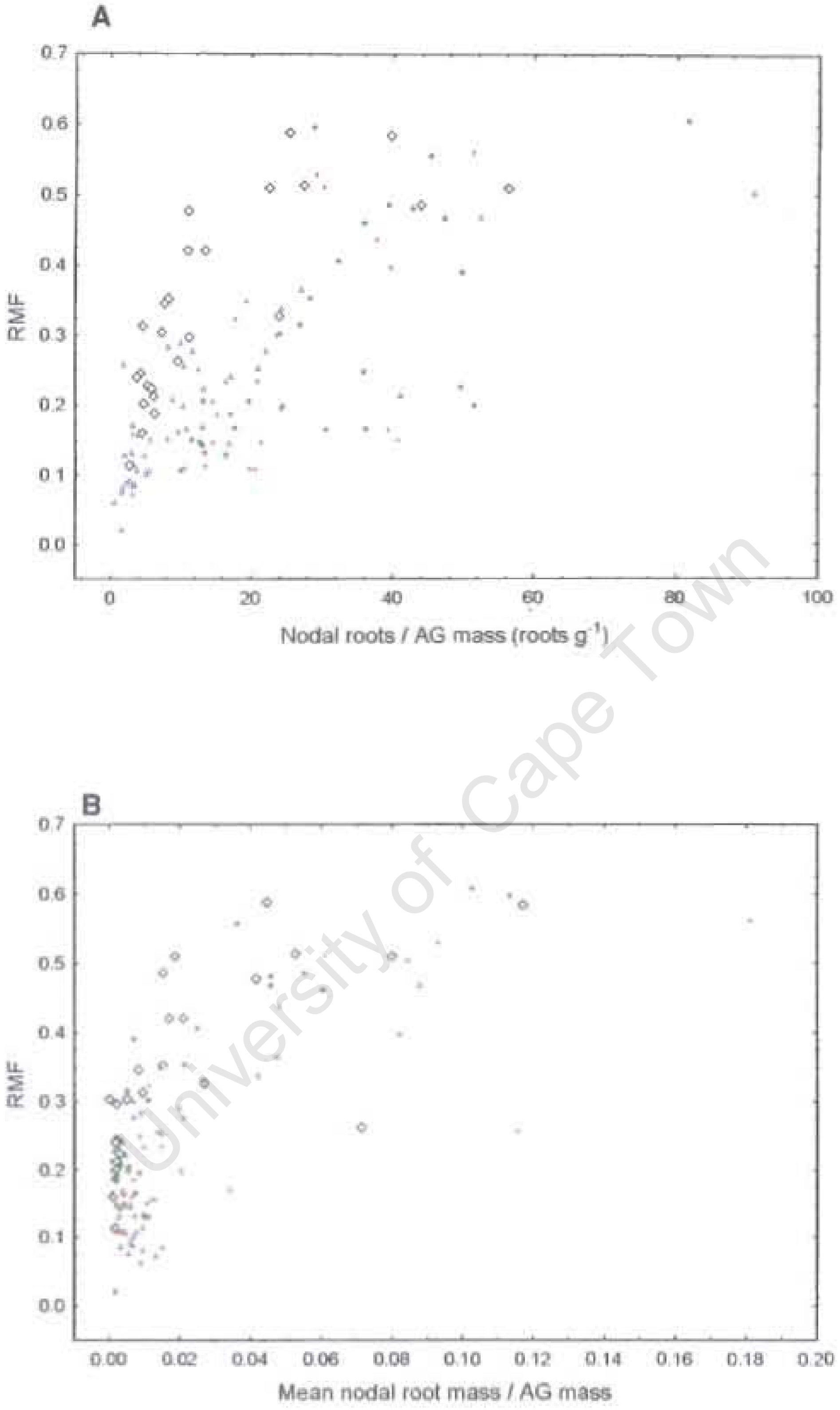


Figure 2.12 The contribution of the number of nodal roots per unit AG mass **(A)** and the mean nodal root mass per unit AG mass **(B)** to changes in root mass fraction (RMF) for *Digitaria:* · , *Urochloa:* ◇ , *Dactyloctenium:* ▵ , and *Sporobolus* + , grown on at least five levels of NH_4 NO_3 in solution culture.

Urochloa were close to those reported for a crop grass species, *Zea mays* (up to 8cm day per day - Cahn *et al.* 1989).

Nodal root SRL (SLR_N)

Increased nodal root elongation would only result in increased nodal root mass if tissue density did not change (i.e. SRL_N remained constant). The GLM ANCOVA model for SRL_N revealed no effect of N supply (**Table 2.11**). Nodal root length did account for some of the variance in the data: SRL_N increased with root length for *Digitaria*, but showed the reverse trend for *Urochloa* and *Sporobolus* (**Fig. 2.13A**). As a result the 'species x length' interaction term of the ANCOVA model was significantly different for *Digitaria* relative to *Sporobolus*, *Urochloa* and *Dactyloctenium*. Taking the effects of length into account, the ANCOVA showed no effect of N supply (non-significant 'species x length x N supply' interactions). Nodal root diameters did not appear to vary from one treatment level to the next either (pers. obs.). This indicates that increases in nodal root elongation did not occur at the expense of nodal root tissue density.

Post-hoc analysis of the ANCOVA model (controlling for the effects of both length and N supply) indicated that the two slowest-growing species had significantly different SRL_N, while *Dactyloctenium* had significantly higher SRL_N, and *Urochloa* significantly lower SRL_N than these two species.

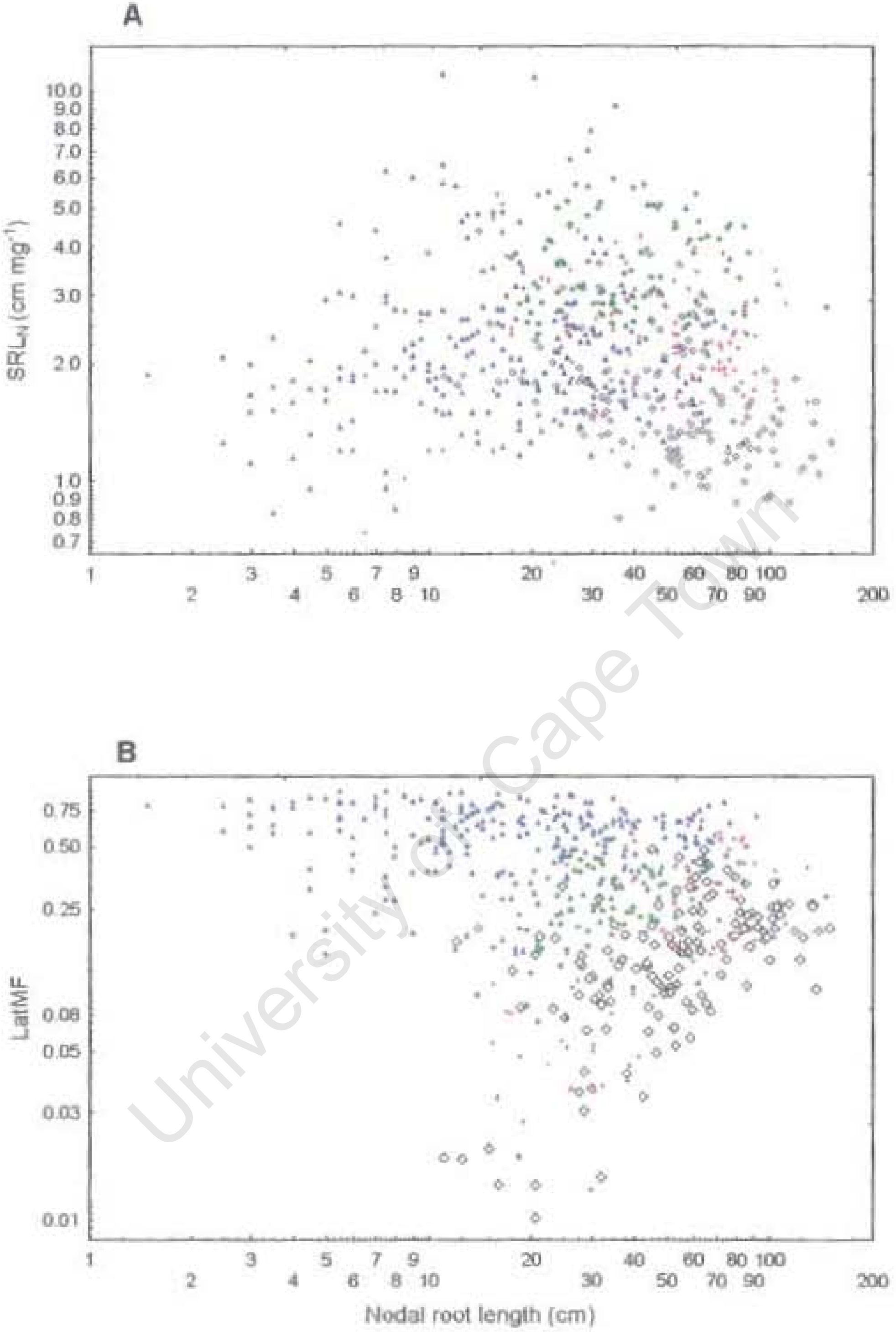


Figure 2.13. Log-log plots of the specific root length of nodal roots (SRL$_N$; **A**) and the mass fraction of nodal roots comprised of lateral roots (LatMF; **B**) versus nodal root length, for *Digitaria*: •, *Urochloa*: •, *Dactyloctenium*: •, and *Sporobolus*: •, grown on an at least five levels of NH_4NO_3 in solution culture.

Table 2.1i. The effect of species, nodal root length and N supply on the specific root length of nodal roots (SRL_N) of the four lawn grass species grown in solution culture. Parameter estimates and standard errors for the GLM 'separate slopes' ANCOVA model fitted to the SRL_N data are shown. B is the co-efficient for ln(SRL_N). Species was the main effect; N supply and ln(length) are covariates. The model was significant: $r^2 = 0.42$, $F_{15,568} = 28.6$, $p < 0.001$. The model was over-parameterized with B set to zero to *Sporobolus*.

Variable	**Species**	**Effect (F = fixed; R = random)**		**SE**	**p**
Intercept		**F**	**1.39**	**0.31**	**0.000**
Species	*Digitaria*	R	0.14	0.38	0.724
	Urochloa	**R**	**-0.91**	**0.33**	**0.005**
	Dactyloctenium	R	0.11	0.42	0.800
	Sporobolus	R	0.00		
N supply		**F**	**-5.08**	**2.25**	**0.024**
ln(length)		F	-0.13	0.08	0.097
Species x N supply	***Digitaria***	**R**	**5.08**	**2.25**	**0.024**
	Urochloa	**R**	**5.09**	**2.25**	**0.024**
	Dactyloctenium	**R**	**5.08**	**2.30**	**0.028**
	Sporobolus	R	0.00		
Species x ln(length)	***Digitaria***	R	-0.13	0.10	0.170
	Urochloa	**R**	**0.26**	**0.09**	**0.003**
	Dactyloctenium	R	0.10	0.11	0.399
	Sporobolus	R	0.00		
N supply x ln(length)		F	1.12	0.61	0.065
Species x N supply x ln(length)	***Digitaria***	R	-1.12	0.61	0.065
	Urochloa	R	-1.12	0.61	0.064
	Dactyloctenium	R	-1.15	0.62	0.065
	Sporobolus	R	0.00		

Lateral root mass fraction (LatMF)

The GLM ANCOVA model for the LatMF data provided a reasonably good fit **(Table 2.12)**. The effect of nodal root length on lateral root production appeared to differ for the mesic versus semi-arid species **(Fig. 2.11B)**. For *Digitaria* and *Dactyloctenium,* LatMF was more or less the same from the length at which laterals started growing onwards (resulting in similar co-efficients in the ANCOVA model). This was not true for *Sporobolus* and *Urochloa*, which produced proportionately little lateral root mass on short roots but increased this proportion as roots grew longer. As with SRL_N, LatMF increases were more or less linear, but showed increasing variance with increasing length). In addition, *Digitaria* roots began producing laterals at a much shorter nodal root length than the other three species.

Taking into account changes in nodal root length, there was no effect of N supply (non-significant 'species x length x N supply' interactions). Post-hoc analysis (controlling for differences in length and N supply) indicated that LatMF was significantly different for each species (*Digitaria* > *Dactyloctenium* > *Sporobolus* > *Urochloa*).

Ramet placement

For *Digitaria* and *Dactyloctenium* clonal foraging for N was not observed, as neither species increased the spacing between ramets in response to low N supply. Branching intensity was highly variable, but showed the reverse trend to foraging, and increased with decreasing N supply, particularly for *Dactyloctenium* **(Fig 2.11C)**. It was observed that most branching for *Digitaria* was from the original crown – primary stolons rarely branched. In contrast, *Dactyloctenium* produced very few primary stolons. Rather, it was the production of secondary stolons from the nodes of primary stolons that resulted in large increases in branching intensity with increasing RGR. Branching angle, measured for *Dactyloctenium* only, showed no changes from one N supply level to the next (data not shown). Changes in stolon internode length for *Dactyloctenium* also showed a 'non-foraging' pattern, with shorter stolon internodes at lower N supply **(Fig. 2.11D)**. *Digitaria* did not show as great a reduction with only a slight increase in mean internode length. Finally, there were no apparent changes in specific culm length (SCL) for either species (data no shown).

Table 2.12. The effects of species, nodal root length and N supply on the mass fraction of nodal roots consisting of lateral roots (LatMF) for the four lawn grass species grown in solution culture. Parameter estimates and standard errors for the GLM 'separate slopes' ANCOVA model fitted to the LatMF data are shown. B is the co-efficient for ln(LatMF). Species was the main effect; N supply and ln(length) are covariates. The model was significant: $r^2 = 0.66$, $F_{15,550} = 70.1$, $p < 0.001$. The model was over-parametized with B set to zero to *Sporobolus*.

Variable	**Species**	**Effect** (F = fixed; R = random)		**SE**	**p**
Intercept		**F**	**-6.94**	**0.58**	**0.000**
Species	***Digitaria***	**R**	**6.50**	**0.60**	**0.000**
	Urochloa	R	**2.01**	**0.67**	**0.003**
	Dactyloctenium	**R**	**5.04**	**0.74**	**0.000**
	Sporobolus	R	0.00		
N supply		F	6.08	5.59	0.277
ln(length)		**F**	**1.22**	**0.15**	**0.000**
Species x N supply	*Digitaria*	R	-6.10	5.59	0.276
	Urochloa	R	-6.21	5.59	0.267
	Dactyloctenium	R	-7.82	5.65	0.167
	Sporobolus	R	0.00		
Species x ln(length)	***Digitaria***	**R**	**-1.28**	**0.16**	**0.000**
	Urochloa	**R**	**-0.49**	**0.17**	**0.004**
	Dactyloctenium	**R**	**-1.07**	**0.19**	**0.000**
	Sporobolus	R	0.00		
N supply x ln(length)		F	-0.03	1.45	0.981
Species x N supply x ln(length)	*Digitaria*	R	0.04	1.45	0.979
	Urochloa	R	0.07	1.45	0.961
	Dactyloctenium	R	0.50	1.46	0.734
	Sporobolus	R	0.00		

Allocation above-ground

Leaf (blade) area ratio (LAR$_B$)

All species showed increased LAR$_B$ in response to increasing N supply, with strong positive correlations between RGR and LAR$_B$ within species (**Table 2.9**). As with RMF, responses varied between species (**Fig. 2.11E**) and responses curves were all significantly different from each other (**Table 2.10**). However, in contrast to RMF patterns, LAR$_B$ responses did not reflect RGR responses. *Urochloa* and *Digitaria*, the fastest and slowest growers, had similar maximum LAR$_B$ which was lower than that of the other two species, although only significantly so for the latter (**Table 2.10**). As a result, there were no correlations between species at optimum N (**Table 2.9**). *Dactyloctenium* had both the highest maximum LAR$_B$, the largest reduction in LAR$_B$, and the sharpest decline in LAR$_B$. *Sporobolus* was intermediate between *Digitaria* and *Dactyloctenium*, and all species converged to similar values at the lowest N supply level.

Components of LAR$_B$

Leaf (blade) mass fraction (LMF$_B$)

Changes in LAR$_B$ could be at least partly explained by changes LMF$_B$ for all species, and strong correlations were found between these two variables within species (**Table 2.9**). However, comparing between species, responses were again different and unique (**Fig. 2.11F**). Response curves were all significantly different from each other, but reflected neither changes in RGR or LAR$_B$. There was no correlation between optimum LMF$_B$ and optimum RGR or optimum LAR$_B$ (**Table 2.9**). *Sporobolus*, the species which does not produce culms had significantly higher maximum LMF$_B$ (**Table 2.10**) and showed the largest reductions in LMF$_B$. Of the other three, the two faster-growers, *Urochloa* and *Dactyloctenium*, achieved a similar maximum LMF$_B$, but for *Urochloa* decrease in LMF$_B$ were more gradual and occurred over a wider range of N supply. *Digitaria* allocated the least to leaf blades and showed the smallest adjustments of LMF$_B$.

Specific leaf (blade) area (SLA_B)

SLA_B, the other component of LAR_B, showed a marked divergence in terms of species responses to N supply **(Fig 2.11G)**. The two mesic species, *Digitaria* and *Dactyloctenium*, had far higher maximum SLA_B than the two semi-arid species, *Urochloa* and *Sporobolus* **(Table 2.10)**, which showed almost no adjustment of SLA_B in response to N supply. Non-linear regressions could not be fitted to the SLA_B data for the latter two species. For *Urochloa*, there was not even a significant linear relationship between SLA_B and N supply, while for *Sporobolus* a weak positive correlation was found **(Table 2.9)**. The response curves for *Digitaria* and *Dactyloctenium* were of a similar shape, but *Dactyloctenium* achieved a higher maximum SLA_B resulting in significant differences between the regressions fitted **(Table 2.10)**. At the lowest N supply level, neither of these species reduced SLA_B to values as low as the mean SLA_B for *Urochloa* and *Sporobolus*. Again there was no correlation between optimum SLA_B and optimum LAR_B (or optimum RGR) when comparing species **(Table 2.9)**.

Above-ground, dead mass fraction (DMF_{AG})

Non-root plant mass that was dead consisted almost entirely of leaf blades and sheaths. There did not appear to be any relationship between RGR and the accumulation of dead material, for the three species for which it was measured, with both the fastest and one of the slowest growing species having similar minimum DMF_{AG} **(Fig. 2.11I)**. As result, optimum DMF_{AG} did correlate with optimum RGR **(Table 2.9)**. However, the fast grower, *Urochloa*, did accumulate the most dead material at all N levels except the highest. At 40mM N, DMF_{AG} increased from the minimum for *Digitaria* and *Sporobolus*. In response to low N supply, DMF_{AG} increased at a similar rate for all three species, although these responses were significantly different **(Table 2.10)**. *Digitaria* accumulated the least above-ground dead material at the lowest N supply level.

Structural organs

Allocation to culms (CMF) showed no clear pattern of change with N supply for *Urochloa* and *Digitaria*, and decreased only slightly for *Dactyloctenium*. Allocation to crowns (CrMF) increased at lower N supply but was also showed large variance (data not shown). *Sporobolus* was an exception to this trend, and showed a very slight decrease in CrMF with decreasing N supply. Changes in allocation to leaf sheaths (LMF_S) mirrored those of LMF_B, with a strong decrease at low N supply. However, reductions were not as severe as those of LMF_B for the two faster growing species. As a result, *Urochloa* and *Dactyloctenium* showed an increase in the fraction of leaves comprised of blades (BMF) at lower N supply (**Fig. 2.11H**).

DISCUSSION

Before discussing the role of the observed changes in biomass allocation in determining the observed changes in RGR, some general problems regarding the ecological and physiological significance of biomass allocation patterns are discussed. Thereafter, the question of what the biomass allocation patterns *per se* can tell us about the N response of these species under natural conditions is addressed.

Interpretation of allocation patterns

Biomass allocation was measured in an attempt to uncover adaptations that enable a species to modify its rate of uptake of a resource when the supply of that resource is reduced. This approach has three potential problems. Firstly, changes in allocation may not be a direct response to changes in resource supply, but rather a result of ontogenetic drift. Secondly, the effect of phylogeny could obscure the importance of allocation changes when comparing between species. Finally, biomass allocation is only a surrogate measure of resource acquisition.

1) Ontogenetic drift

Ontogenetic drift refers to a change in allocation patterns which occurs as a plant grows larger, and may lead to the erroneous conclusion that a treatment effect caused changes in allocation, while the real reason is that the treatment effect simply slowed growth and delayed the development of an allocation pattern found at higher treatment level (Coleman *et al.* 1994; Coleman and McConnaughay 1995). However, while ontogenetic drift may obscure the physiological mechanism responsible for differences in allocation, allocation patterns observed at a given plant size are still of ecological significance, as argued by Coleman *et al.* (1994) and Poorter and Nagel (Coleman *et al.* 1994; 2000). It is the actual relative sizes of organs which influences plant resource acquisition at any given time, and not their potential relative sizes. For example, suppose that ontogenetic drift was a factor in this experiment, and that differences in RMF were simply a result of differences in RGR and not plant N concentration. A *Urochloa* plant growing at the mean RGR calculated for 0.016 mM N supply, would take 189 days to reach the mean mass of those at the highest level. This is about three quarters the length of the growing season from the habitat where it was collected. This would mean that if a plant had this low supply of N in its habitat, it would miss out on the benefits of the lower RMF obtained by a plant receiving the highest N supply for almost three quarters of the growing season (potential benefits would include anything associated with the actual size of above-ground parts, such out-shading of competitors or enhanced tolerance of defoliation).

'Allometric analysis' can be used to test for ontogenetic drift. This involves linear regression of organ mass against whole plant mass. Differences in organ size are then only considered significant if the slope of the this regression differs significantly for plants from different treatment levels (Poorter and Nagel 2000). However, this technique was not employed because it is only appropriate when plant sizes are similar. In this study, differences in plant size at harvest were so large that regression lines would have to be extrapolated well beyond the range of plant sizes in each treatment level. And as it is possible that organ sizes scale isometrically (i.e. in a non-linear fashion) with whole plant sizes (for any given treatment level), this would not be acceptable. In addition, the small sample sizes and large variance within samples make it likely that genuine changes in allocation would be overlooked. Finally, as many of the grasses in this study began to flower at sizes smaller than at harvest (even at the lower N supply level), ontogenetic drift may have ceased well before harvest.

2) **Phylogeny**

Allocation patterns are often compared across a diverse range of species, without taking the (potential) influence of phylogeny into account. The use of 'phylogenetically independent contrasts' adjusts between-species comparisons according to the degree of 'relatedness' between a pair of species and thus controls for phylogeny (Villar *et al.* 1998). For example, Antunez *et al.* (2001) found that the generally accepted pattern of deciduous trees having a higher RGR than evergreen trees does not hold when tested using phylogenetic contrasts, as only five of the eight deciduous-evergreen species pairs tested showed the expected relationship.

The incorporation of phylogeny is a relatively new approach to allocation studies, and only one such study involving grasses was found (Villar *et al.* 1998). Villar *et al.*

found that including the effect of phylogeny had only a small influence on allocation patterns, and strengthened established patterns for almost all variables measured for 20 grass species of the genus *Aegilops*. In this study, the use of only four species is too few to employ phylogenetic contrasts. However, it is worth noting that while the four species used are from two different subfamilies, there were no consistent patterns between the sub-families pairs (*Digitaria* and *Urochloa* versus *Sporobolus* and *Dactyloctenium*).

3) Biomass allocation as a surrogate

Allocation patterns are based on organ mass, but organ mass does not necessarily determine resource acquisition. The uptake of any resource is the product of the area of absorption surfaces and the rate of uptake per unit absorption area (specific uptake rate), and organ mass is at best a surrogate measure of these two traits. Furthermore, allocation is a static variable – a measure of net allocation – and does not take rates of tissue turnover into account. Biomass will therefore underestimate differences in actual allocation when comparing two samples if plants in one have higher tissue turnover. However, as in the case of ontogenetic drift, it is organ size *per se* (not processes which create organ size) that affects resource flows between a plant and its environment and is therefore of ecological significance.

Physiological significance of observed patterns

Taking the above problems into account, as well as results from similar studies, the likely significance of the observed changes in biomass allocation for growth are discussed below.

Allocation to root

RMF patterns

Allocation patterns conform to the 'functional equilibrium' model proposed in the early 1960's by R. Brouwer, which predicts increased allocation to the organs responsible for acquiring resources which are most limiting (Poorter and Nagel 2000). The observed increases in RMF, in response to a decreased supply of a nutrient, has been found in the vast majority of studies (recently reviewed by Reynolds and D'Antonio 1996; Poorter and Nagel 2000). The very low values of RMF for the two slower-growing species, at optimum N supply, is not unusual: Crick and Grime (1987) report an RMF as low as 0.083 for a stoloniferous grass species, while two graminoid species from the Serengeti ecosystem had a RMF of approximately 0.1, when grown in solution culture (Fig. 3 in McNaughton and Chapin 1985). However, maximum values of RMF at low N supply were generally higher those that reported for eight C_4 grass species from desert habitats when subjected to water stress in a sand culture experiment by (Fernandez and Reynolds 2000).

The re-increase in RMF at higher level suggests the onset of toxicity at N supply below 40mM for *Digitaria* and *Sporobolus*. This pattern has been shown in other gradient experiments for both shrub and grass species (Ruess *et al.* 1983; Redente *et al.* 1992).

Components of RMF

Increased RMF could result from an increased rate of nodal root production per unit above-ground (AG) growth, or an increase in the mean growth rate of the nodal roots produced, per unit AG growth (or both). Assuming rates of root and shoot had reached a steady state (both in terms of production and turn-over), 'number of nodal roots / AG mass' should have provided a measure of the relative rate of nodal root production, and 'mean nodal root mass / AG mass' should have provided a measure of the relative rate of nodal roots growth. The observed relationships between these two variables and RMF showed only slight differences between species. It appears that increased allocation to root resulted from an increase in the rates of both nodal root production and nodal root growth for all four species. However, the large variance in the data, particularly at high RMF values, suggests that the above assumption of a steady state is not entirely valid.

The only notable distinction regarding nodal root growth was that the slower-growing species, *Sporobolus*, apparently increased rates of nodal root growth (relative to AG growth) with decreasing N supply while the two faster growing species. *Urochloa* and *Dactyloctenium* did not (**Fig. 2.11B**). While analysis of root elongation is not common in these types of studies, this response has been reported for at least one other species (*Festuca arundinaceae* - Gastal and Durand 2000).

Nodal root turnover

As root mortality was not measured, it cannot be said from the results whether changes in root turnover between N supply levels affected changes in RMF. *Sporobolus* appeared to have a higher ratio of dead-live roots at harvest (particularly at low N supply) indicating a higher turnover. If root production rates were similar to for all species (as estimated from 'number nodal roots / AG biomass'), then RMF may have underestimated allocation to root for this species. In addition, this suggests shorter nodal root longevity, and thus contradicts the pattern of slower-growing species have longer-lived tissues, proposed by Campbell and Grime (1989) and supported (for roots) by *in situ* measurements (Schlapfer and Ryser 1996; Fransen and de Kroon 2001; van der Krift and Berendse 2002).

Ontogenetic drift

It is possible that large range in RMF values found is an artefact of ontogenetic drift. RMF often declines with increasing plant size for herbaceous species (Poorter and Nagel 2000). The large variation in final biomass between treatment levels makes this a potential problem. A recent meta-analysis by Niklas and Enquist (2002) also casts doubt on the validity of wide ranges in RMF. They show the growth of roots scales proportionately to AG growth for a huge range of species, with very little variance. This means that RMF should be the same for all species (grown under any resource supply) - Niklas and Enquist calculate an average value of just over 20%. However, such remarkable uniformity in allocation to roots is in stark contrast to the other meta-analyses investigating allocation patterns (Reynolds and D'Antonio 1996; Poorter and Nagel 2000). Furthermore, experiments which maintain constant internal N concentrations by supplying N at exponentially increasing rates have shown a strong correlation between N supply and RMF (Freijsen *et al.*; Boot *et al.* 1990a; Agren and Bosatta 1996), while the meta-analysis by Garnier (1998) indicates that this pattern holds even when plants are grown with a less strict regulation of N supply. Therefore, it is maintained that the large ranges of RMF observed here were in fact a result of the plants actively increasing the growth of roots relative to shoots.

Effect of defoliation

While many plant level experiments report that clipping reduces allocation to roots (Walker *et al.* 1999; Dawson *et al.* 2000), even for grazing-tolerant species (Ruess *et al.* 1983; Jaramillo and Detling 1988), there are exceptions (Dawson *et al.* 2000). In addition, community level studies report that grazing has either no effect on root production (McNaughton *et al.* 1998) or actually increases it (Frank *et al.* 2002). For this study, whether clipping altered allocation to roots is only of interest if this occurred to a different degree on different treatment levels. For example, Ruess *et al.* (1983) found that clipping reduced or even reversed increases in RMF, in response to decreased N supply, when *Kyllinga nervosa* was clipped weekly. Unfortunately, the data collected provide no insight regarding this potential experimental error. No experiments were found in the literature which actually measure the response of RMF to both N supply and defoliation. However, as was the case here, the studies by McNaughton *et al.* (1983) and Hamilton III *et al.* (1998), report increases in RMF in response to decreased N supply, despite frequent clipping, for two grazing-tolerant graminoids.

Finally, clipping may have hindered lateral root growth. Dawson *et al.* (2000) found that clipping prevented a significant proliferation of laterals in response to decreased N supply for *Festuca ovina* (although not for *Lolium perenne*). Also, Ruess (1984) found that browsing reduced fine root production in a forest ecosystem.

RMF and N uptake

Presumably increases in RMF at low N supply minimized growth reductions by limiting reductions in the rate of N acquired (per unit growth) by the entire root system. Such increases in allocation to root (relative to the rest of the plant) could have altered N acquisition by:

1. producing a directly proportional increase in root surface area (i.e. changing root area without changing root tissue density), or
2. producing a 'more than proportional' increase in root surface area by decreasing tissue density as well, and / or
3. increasing the rate of N uptake per unit surface area (specific uptake rate) - at least one study shows that a grass species (wheat) can adjust both root area and specific uptake rate (van Vuuren *et al.* 1996).

Surface area

Each of the above mechanisms is difficult to measure, and this was not attempted in this study. However, as no significant changes were found for SRL_N or LatMF, one might conclude that all four species adjusted root surface area without adjusting the density of root tissues (note that provided that the tissue density of lateral roots is less than or equal to that of nodal roots, an increase in LatMF would produce a decrease in the average tissue density of the entire root system, as lateral roots have a smaller diameter than nodal roots). A lack of change in root tissue density in response to N supply has also been found by Boot and Mensink (1990) who measured specific root area (the area of root per unit weight) directly. In addition, Boot and Mensink (1990) found that changes in root-shoot ratio for five species were similar to changes in 'leaf area : root length' ratios (root length being a surrogate measure for root area), suggesting that changes in root area were in direct proportion to changes in root mass. On the other hand, an increase in lateral root production in response to nitrate supply - which indicates a decrease in tissue density of the entire root system - has been reported for maize (Granato and Raper 1989).

When comparing changes in variables such as SRL_N or LatMF, a problem similar to that of ontogenetic drift arises, in that comparing species are different growth rates introduces the danger of an interaction between growth rate and allocation. In other words, a slow-growing plant may not have had enough time to make adjustments equivalent to that of a faster growing plant by the time of harvest. An analytical model by Fransen *et al.* (1999) shows how morphological changes at the root level can be misinterpreted if plants differing in RGR are compared at a common time rather than a common biomass. Therefore it is possible that plants at lower N supply may have eventually changed the tissue density of their nodal roots by the time they reached the size at which plant were harvested at high N supply. However, as with ontogenetic drift, the observed changes are still of ecological significance.

One potentially important aspect of allocation which was not measured is allocation to root hairs. An increase in root hair production would achieve a disproportionate increase in root area, and their potential contribution to enhanced nutrient acquisition has long been stated (Clarkson 1985). Although the proliferation of roots hairs is considered to be more beneficial for the uptake of slow-moving ions such as phosphate (Lambers *et al.* 1998), root hair density has been shown to increase in response to N stress (Robinson and Rorison 1985; Boot 1990).

Consideration also needs to be given to the fact that by adjusting architecture, it is possible for a plants to change the surface area of its root system without changing allocation to roots at all (Fitter 1994). Berntson (1994) showed that branching (measured as grid intercept fractal dimension) changed in response to uniform fertilization for two birch species. A certain amount of architectural change was in fact achieved by the four species in this study, as the number of nodal roots relative to AG biomass increased with RMF. This indicates that in response to a reduced supply of N, the plants produced more nodal roots per unit of biomass allocated below-ground. This has also been found, although in response to phosphorus (P) starvation, for *Zea mays* (Mollier and Pellerin 1999). It is quite possible that similar changes in branching patterns of lateral roots were overlooked.

Specific uptake rate

Whether changes in specific uptake rates also occurred in this study cannot be said. Few studies investigating specific uptake were found in the literature, and only one relating to N uptake (Ruess *et al.* 1983). However, most of these indicate that decreases in specific uptake in response to decreased nutrient supply can be proportionately less than expected. Ruess *et al.* (1983) found that N uptake (per unit root length) was only reduced by about half when N supply was reduced by a factor of 10. Furthermore, this (relatively) high uptake rate at low N supply resulted in higher plant N concentrations for clipped plants, as compared to unclipped controls, even though clipping prevented increases in RMF at low N supply (McNaughton and Chapin 1985). McNaughton and Chapin found that kinetic parameters of P uptake

(V_{max} and K_m) were not affected when a grazing-tolerant species was grown in solutions containing 10 as opposed to 100:M P. Also, a recent simulation model indicates only a 2-fold change in uptake kinetics in response to changes in nutrient solution concentrations for lupins (Dunbabin *et al.* 2002). On the other hand, other modelling studies suggest that ion uptake parameters have no influence on nutrient uptake rate and are simply determined by external nutrient supply (Clarkson 1985).

Allocation above-ground

An increase in allocation to roots necessarily decreases the relative biomass of AG parts. Therefore, increased allocation to roots would only mitigate the effects of reduced N supply on growth if a plant is able to reduce rates of photosynthesis less than in proportion to decreases in AG biomass. This can be achieved by allocating more biomass to assimilatory organs, relative to the non-assimilatory (structural) AG organs. Furthermore, photosynthesis by the assimilatory organs can be increased (per unit biomass), by increasing light capture per unit assimilatory area or increasing the amount of assimilatory area per unit biomass (or both). Leaf sheaths and stolons in the species involved in this study were mostly green, and appear to have both an assimilatory and structural function. However, although rates of photosynthesis occurring in these organs is unknown, it is assumed that the presence of large amounts of structural material inhibits their photosynthesis to rates negligible compared to that of leaf blades. Further discussion regarding 'the assimilatory organs' is therefore limited to leaf blades.

Allocation to leaf blade

Unlike root allocation, assimilatory area was measured for leaves, and the functional significance of increased allocation to leaf blades can be addressed directly. It is therefore possible to determine whether that changes in biomass allocation to leaf blades resulted in directly proportional changes in leaf blade area, or whether changes in tissue density occurred as well.

LAR_B

The observed decreases of LAR_B, and the related positive relationship between LAR_B and RGR (within species), is a common response to decreased nutrient supply. (Poorter and Remkes 1990; Poorter and Nagel 2000). While the lack of a significant correlation between LAR_B and RGR (comparing between species) is unusual, it has been reported: Villar *et al.* (1998) found only weak, non-significant relationships between RGR and LAR (whether or not they controlled for phylogeny); Ghannoum *et al.* (2001) found no correlation between biomass and accumulation and LAR for a number of NADP-ME C_4 grasses.

LMF_B

Underlying the adjustment of LAR_B was the decrease in LMF_B and there was a strong correlation between allocation to blade and LAR_B – again a widely reported pattern in response to nutrient supply (Poorter and Remkes 1990; Fichtner and Schulze; Poorter and Nagel 2000). At least part of this reduced allocation resulted from a reduction in the rate of elongation of leaf blades (part 1 of this section), which is a common response to reduced N supply for grasses (Cruz and Boval 2000).

SLA_B

Changes in LAR are usually accompanied by changes in tissue density as well as, with decreases in LAR resulting from both reduced biomass allocation to leaf and increased tissue density - (measured as SLA, Poorter and Remkes 1990; Villar *et al.* 1998; Poorter and Nagel 2000). The lack of adjustment of SLA_B for *Urochloa* and *Sporobolus,* and the resulting lack of a correlation between SLA_B and RGR, contrasts with most other studies cited regarding grass allocation patterns, Meziane and Shipley (1999) being an exception. However, as Meziane and Shipley point out, studies which have found a significant correlation between RGR and SLA are conducted at low light levels – at higher light levels, NAR has a far stronger influence on RGR (see below). It is also possible that the apparent lack of changes in tissue density are an artefact produced because SLA does not take differences in leaf thickness into account. This is unlikely, however, as van Arendonk and Poorter (1994) found that for 14 species of grasses, SLA correlated with tissue density but not with leaf volume per unit area.

In addition to the lack of within-species correlation between SLA_B and RGR mentioned above, it is unusual for a slower-growing species (in this case *Digitaria*), to have a higher SLA_B than a faster-grower (*Urochloa*), regardless of N supply (Boot and Mensink 1990; Fichtner and Schulze; Niemann *et al.* 1992; Elberse and Berendse 1993; van Arendonk and Poorter 1994). However, almost all the studies which do show a positive correlation between RGR and SLA_B involve a small sample of C_3 grasses from the temperate areas western Europe. The only exception found being a meta-analysis by Craine *et al.* (2001), which was conducted in North America, and involved a larger sample size and included some C_4 species. In contrast, Villar *et al* (1998) found no significant correlation between SLA and RGR for Mediterranean grasses. Also, Ghannoum *et al.* (2001) found no correlation between biomass accumulation and SLA for a group of NADP-ME C_4 southern hemisphere grasses, and only a weak correlation for NAD-ME species. Therefore, the generally accepted relationship between SLA and RGR may not be relevant for the relatively less studied C_4 sub-tropical species, such as those used here.

Leaf turnover

Since LMF_B at any given time is a function of the rate of both production and death of blade mass (relative to the rate of growth of the rest of the plant), actual allocation to leaf blades is not necessarily equal to LMF_B. For all three species for which it was measured, DMF_{AG} increased with decreasing N supply, resulting in the significant negative correlations between DMF_{AG} and LMF_B (within species). Decreases in LMF_B may therefore have resulted from increased leaf mortality rates alone. If this was the case, then the convergence of DMF_{AG} to similar values at optimum N for *Digitaria* and *Urochloa*, with higher values for *Sporobolus*, would indicate that leaf longevity was lower or similar for the slower-growing species (*Digitaria* and *Sporobolus*) than for the faster-grower (*Urochloa*). A similar pattern was found for C_3 grass species by Schlapfer and Ryser (1996) who report that the faster-growing species had a lower dead:live ratio (for AG parts).

Specific uptake

If one assumes that light assimilated during photosynthesis is absorbed uniformly across all leaf area, then the amount of photosynthetically active radiation (PAR) assimilated per unit leaf area (the 'specific uptake rate' for PAR) is the same as the net assimilation rate (**NAR** – the rate of increase in biomass per unit increase in leaf area). While this variable was not measured, its magnitude can be inferred where a change in area does not produce changes in growth (as, by definition, RGR = LAR x NAR - Hunt 1982).

The strong within-species correlations suggest that adjustments in LAR, rather than NAR, were responsible for most of the change in RGR, a pattern observed in the majority of nutrient addition experiments (Fichtner and Schulze 1992; Poorter and Nagel 2000). In fact, many have found that almost all variation in RGR is a result of changes in LAR, with non-significant effects of NAR (Boot and Mensink 1990; Poorter and Remkes 1990). However, changes in both LAR and NAR have been shown to contribute to changes in RGR for both grass species (Oesterheld and McNaughton 1988; Poorter and Nagel 2000), and a broader range of angiosperm species (Meziane and Shipley 1999). This appeared to be the case in this study, at least for the faster-growing species (*Urochloa*), as although LAR was strongly correlated with RGR, it showed only a gradual increase at higher N supply. Increased NAR is therefore likely to be responsible for the increases in RGR at higher N supply. This implies that *Urochloa* suffered large reductions in NAR with decreasing N supply, while slower-growing species were able to maintain more constant, albeit lower, NAR. Other studies lend some support to this idea, with faster-growing species showing a stronger correlation between RGR and NAR (Boot and Mensink 1990; Boot *et al.* 1990b).

At 40mM N supply the increased allocation to roots was accompanied by reduced LMF_B for *Digitaria* and *Sporobolus,* but LAR_B (and RGR) was maintained. This means that SLA_B must have increased, but the data do not show this (**Fig 2.11G**). This suggests an error in the measurement of either SLA_B or LAR_B (if the latter, then increased NAR must have contributed to RGR for these slow-growing species as well).

Allocation to structural organs

The patterns of increased allocation to crown and culm with decreasing N supply were most likely the result of a (relative) increase in tiller mortality at lower N supply.

It was observed that young tillers at the lower N levels often died before producing more than one or two juvenile leaves, particularly if clipped. When this occurred, leaves became standing dead, but the base of the tiller, and any culm which had developed, remained alive and thus contributed to crown / culm biomass at harvest. A pattern of increased crown mass fraction at lower N supply was also reported for *Kyllinga nervosa* (Ruess *et al.* 1983), although only when N was supplied as nitrate.

It was surprising that, with the exception of *Dactyloctenium*, allocation to sheaths did not decrease relatively more than to blades as N supply decreased (as indicated by constant BMF, the fraction of total leaf mass consisting of blades). This may indicate that these organs do make a significant contribution to whole-plant photosynthesis, as increases in BMF would then not help to reduce the deleterious effect of reduced allocation to leaf blade area. Measurements of net photosynthesis of sheaths (and culms) are needed to investigate this. Alternatively, biomechanical constraints may have prevented increases in BMF (i.e. BMF may have been maximal at optimum N supply). However, from personal observation, the most likely explanation for the BMF pattern reported is differences in blade and sheath longevity. While many blades died before reaching the 'adult' stage, the attached sheathes remained alive far longer. As a result, leaves on the lower N supply levels consisted of a many dead blades but mostly live sheaths, while at higher N supply, most blades and sheaths were still alive at the time of harvest. Thus even if allocation to blades was increased with increasing N supply (relative to sheaths), sheath mass remained relatively constant.

Allocation to inflorescences

Differences in inflorescence production offered little insight regarding growth responses, as the species which flowered did so across a number of N supply levels. The patterns proposed by Chapin III (1980), that plants with slower growth rates flower less, and that nutrient stress delays reproduction, were not supported. A similar result was found for *Kyllinga nervosa,* which flowered at fairly low N supply (Ruess *et al.* 1983), while Bertiller *et al.* (2002) found no effect of N on allocation to panicles for a species of *Poa*, in an *in situ* study. The lack of flowering shown by *Dactyloctenium* may have been a result of the length of the photoperiod in the growth chamber – it has been observed in HUP that *Digitaria*, *Urochloa* and *Sporobolus* begin flowering in the spring and continue right through the growing season, while *Dactyloctenium* only begins flowering in about mid-December.

Ecological significance of observed patterns

As with RGR, it has been suggested that differences in biomass allocation patterns *per se* reflect differences in the nutrient demands of species. Whether the allocation patterns observed in this study indicate that the four species involved are adapted to exploit habitats of different N supply is discussed below.

Allocation to root

Optimum RMF

It has been proposed that species which dominate nutrient-rich habitats have not only greater maximum RGR but also lower RMF at optimum N (Elberse and Berendse 1993). As with the relationship between RGR and habitat fertility, there are a number of reasons to doubt this. Firstly, species with greater maximum RGR do not necessarily have lower optimum RMF. In this study, the two slowest-growing species, *Digitaria* and *Sporobolus*, achieved the lowest RMF at optimum N supply, and this pattern has also been found in other multi-species studies (Boot and Mensink 1990). Secondly, the relationship between optimum RMF and habitat fertility enjoys little empirical support. While Elberse and Berendse (1993) found a correlation between RMF and Ellenberg N, Campbell *et al.* (1991) did not. In addition, Villar *et al.* (1998) found a strong correlation between habitat rainfall and RMF. This suggests that adjustments in root allocation could be as much an adaptation to N supply as water supply.

RMF plasticity

A more widely accepted pattern is that species from nutrient rich habitats show greater morphological plasticity in response to nutrient availability, at least in terms of allocation to root (Grime 1994). Evidence to support this comes from Boot and Mensink (1990) who found larger changes in RMF (in response to N supply) for faster growing species, Elberse and Berendse (1993) who found that the correlation between RMF and Ellenberg N only became significant with fertilization; and the meta-analysis by Reynolds and D'Antonio (1996) which showed that with species with a higher root:shoot ratio at low N supply had a significantly larger reduction of root:shoot at high N. However, the meta-analysis of Garnier (1998) found no differences in RMF plasticity between species of different growth rate or growth form. Note that Garnier's method of analysis was superior to that of Reynolds and D'Antonio, as it used a whole plant weight ratio (the same measure as RMF) as a measure of root allocation, rather than root:shoot ratio (which excludes structural organs), and it controlled for the problem of comparing experiment in which plants were grown at different levels relative to their optima (by means of standardizing data with average RMF). Therefore, the differences in RMF plasticity reported in this study cannot be taken as evidence that the four species tested are adapted to habitats differing in nutrient supply.

The results of Garnier also showed that the relationship between plant N concentration (PNC) and RGR is linear, but the relationship between PNC and RMF is curvilinear with RMF increasing most rapidly at lower levels of PNC (again, this was previously predicted analytically by Agren and Bosatta 1996). These two patterns explain why faster-growing species should not actually have greater allocation plasticity: while a faster-growing species will experience a greater reduction in PNC, from optimum to minimum N supply, this will result in only a negligible increase in RMF adjustment (relative to a slower-growing species). In this study, the lack of a relationship between maximum RGR and total adjustment in RMF corroborate the findings of Garnier.

Lateral root allocation

Controlling for the effects of root length and N supply, SRL_N was not higher for the two species common in more arid environments, as has been found for the SRL of the main root axis for a range of perennial species (Nicotra *et al.* 2001). However, Nicotra *et al.* found the reverse pattern when analysing the SRL of the entire root system. If one assumes that lateral roots have higher SRL, this means that of the species studied by Nicotra *et al.*, those from drier areas had proportionately less lateral roots. Such a pattern is supported by the analysis of the LatMF data here, suggesting that allocation to lateral roots is a reflection of habitat water availability rather than fertility.

Allocation to leaf

LMF

As with allocation to root, between species differences in LMF have been correlated with either directly with habitat fertility (Elberse and Berendse 1993), or with maximum RGR on the assumption that maximum RGR represents habitat fertility (Boot and Mensink 1990; Villar *et al.* 1998). If this were a universal pattern, it would indicate that *Urochloa* and *Sporobolus* are adapted to a high supply of N, as they showed the largest maximum LMF_B. However, as with RGR and RMF, the proposed relationship between LMF and habitat fertility is supported by only a few studies involving only a handful of species. Considering that *Urochloa* and *Sporobolus* showed such different responses to N supply in terms of RGR, but both occur in semi-arid environments, it is suggested that plasticity in LMF (as with lateral root mass) reflects differences in water supply rather than nutrient supply, in the habitats of the four species tested.

Leaf blade plasticity and water availability

Tissue density is generally higher for plants from nutrient poor habitats, a pattern shown by a large meta-analysis by Craine *et al.* (2001), and by N addition experiments (Boot and Mensink 1990; Fichtner and Schulze; Elberse and Berendse 1993). However, the lack of a relationship between and RGR (between species) in this study shows that differences in SLA_B did not contribute to RGR responses to N supply for all species. There is therefore little reason to believe this variable provides a reflection of habitat fertility.

A more likely explanation for the SLA_B pattern in this study is again proposed to be differences in habitat water availability. Semi-arid species such as *Urochloa* and *Sporobolus* may maintain a large amount of structural leaf material to facilitate low leaf water potentials and thus water uptake in dry soils. The study of Villar *et al.* (1998), which also found a lack of correlation between RGR and SLA, included many species from the Mediterranean, where winter drought stress is common. In the study of Fichtner and Schulze (1992) only some of the European C_3 species used increased SLA in response to N, perhaps due to differences in tolerance of drought stress. In another study in which two European C_3 species showed no adjustment of SLA in response to N supply, Boot and den Dubbelden (1990) found no increase in transpiration (at the leaf level) with increased N supply, which resulted higher whole-plant water use efficiency. Perhaps a lack of adjustment of SLA at high N supply is an adaptation to reduce transpiration per unit leaf mass, with increases in NAR being used for faster growth. A small amount of support for this idea comes from van Rensburg *et al.* (1999). They found that *Themeda triandra*, a bunch grass with a wide distribution in both semi-arid and mesic areas, had significantly lower SLA for populations from semi-arid areas. On the other hand, of the eight species of C_4 desert grasses used by Fernandez and Reynolds (2000), the species which showed the smallest in RGR in response to decreased water supply also showed the largest adjustment in SLA. Also, changes in SLA (in response to N supply) have been reported for both lawn and bunch C_4 grasses from semi-arid areas of the Serengeti (Wallace *et al.* 1984). Wallace *et al.* also found that increased N supply increased estimates of whole plant water loss for the two bunch grass species.

To conclude this part of section 3, changes in biomass allocation do appear to have mitigated the effects of reduced N supply on RGR reduction for the four lawn grasses involved in the solution culture experiment. The differences in RGR responses (part 1) were reflected, with different species showing different patterns of allocation adjustment in response to N supply. Significant differences were found in terms of allocation to root, allocation to leaf blade, and the amount of assimilatory area produced per unit of root / shoot mass, although many of these contradict patterns commonly reported in the literature. Direct measures of assimilatory area (for roots), specific uptake / assimilatory rates (for roots and shoots) and rates of tissue turn-over would have enabled a better assessment of the physiological significance of the observed allocation changes. Regardless, the allocation patterns clearly show that the four species involved differ in the way they acquire and utilize N. Finally, no inferences could be made between the allocation patterns *per se*, and N supply of the habitats were these species occur, as general patterns reported in the literature regarding such relationships are too weak to be useful.

PART 3

EXTRAPOLATION

Parts 1 and 2 concluded that the four lawn species tested show different responses to a fairly uniform supply of N. However, this overall result should only be extrapolated to natural conditions with considerable caution, particularly as no clear patterns were found in the literature regarding RGR and biomass allocation responses to N supply, and N responses under natural conditions. To end this section and the chapter, a number of factors which may alter the observed between-species differences under realistic ecological conditions are discussed.

Growth medium

Solution culture provides less physical support, but also allows for unhindered root elongation, as compared to soil. Whether this affected growth differently at different levels of N supply, or at all, is unknown. It is interesting though that Walker *et al.* (1999) found no differences in root branching for two grass species grown both in sand and solution culture.

Spatio-temporal variation in N supply

Perhaps of far more relevance than physical differences, solution culture provides a far more uniform supply of nutrients than soil. Variation in litter inputs, rhizodeposition, microbial activity, soil moisture, soil adsorbtion properties, uptake by roots of neighbouring plants and leaching are all likely to contribute a very different supply of N (and other nutrients) in soil. As mentioned in the introduction, many studies provide evidence of the effects of mainly spatial, but also temporal, variation of nutrient supply on grass growth.

Foraging at the scale of nodal roots

Where increased growth results from localized root proliferation, it is clearly due to a relative increase in lateral root growth (Caldwell *et al.* 1991b; van Vuuren *et al.* 1996; Cui and Caldwell 1998; Fransen *et al.* 1998; Hodge *et al.* 1999a, b). However, the fact that none of the species in this study showed an ability to proliferate laterals cannot simply be accepted as evidence of a lack of a foraging ability for a number of reasons.

1) It is possible that variation in N supply down the length of a nodal root is needed to initiate proliferation, rather than variation across entire root systems (the observation that proliferation was variable down the length of many *Sporobolus* nodal roots would support this).

2) In some cases proliferation has been shown to be ineffectual for enhancing N uptake (Fransen *et al.* 1998; Fransen and de Kroon 2001), while Caldwell (1994) found poor relationship between grass root density and soil nitrate. It seems that other mechanisms – a change in lateral root architecture (Hodge *et al.* 1999b), or an increase in specific uptake rate (Campbell and Grime 1989; van Vuuren *et al.* 1996; Fransen *et al.* 1999; Hodge *et al.* 1999a) – can produce patch exploitation without root proliferation. And as root areas and uptake rates were not measured in this study, the possibility that the species involved here have such adaptations cannot be excluded.

3) The ability of *Sporobolus* to maintain rates of nodal root elongation at low N supply is a potential foraging adaptation. Simulation models suggest that root elongation is an important factor affecting total uptake (Clarkson 1985). Thus *Sporobolus* may be increasing its ability to exploit a patch faster than competitors by maintaining high rates of elongation. In addition, higher elongation rates through patches of low N supply would result in a greater number of N-rich patches reached, relative to a species which decreases elongation in response to decreased N supply. A more practical measure of root elongation would be required to investigate such ideas, as the method used was not practical. The method used by Lecompte *et al.* (2001) has promise, as it allows for prediction of elongation rates from static variables, making it applicable for field measures as well. They show a strong linear relationship exists between the length of the apical unbranched zone (LAUZ) of a root and its elongation

rate; while the product of LAUZ and root apical diameter was a good predictor of the growth rate of laterals on an axis.

4) The presence of roots of neighbouring plants may inhibit access to nutrient-rich patches, as plant root growth has been shown to be inhibited by the presence of roots of other species (Caldwell *et al.* 1991a). Huber-Sannwald *et al.* (1998) showed that rhizome branching in patches of high nutrient availability is severely inhibited, for one species, by the presence of roots of certain bunch grass species – and this resulted in reduced gains in biomass achieved from exploiting the patches in the absence of competition. Adaptations to exploit N rich patches could therefore involve monopolization of patches (by means of secreting of chemicals which inhibit the growth of other plant's roots) rather than rapid exploitation of patches.

Foraging at the scale of ramets

If spatial variation in N supply occurs at a scale greater than that of nodal roots, then the stoloniferous species might be expected to show plasticity in ramet placement, as this would enable the placement of more nodal roots in nutrient rich patches. However, a review by de Kroon and Hutchings (1995) concluded that while plasticity in stolon or rhizome growth may enable foraging for light, it is not important for nutrients. In terms of stolon plasticity, that would be appear to be the case here – only the maintenance of a similar stolon internode length by *Digitaria* (**Fig. 2.11D**) might be of foraging significance. Surprisingly, this response differs from that shown by *Cynodon dactylon*, a species of remarkably similar morphology to *Digitaria* and also common on heavily-grazed areas of southern Africa (Dong and de Kroon 1994). Dong and de Kroon found a doubling of internode length in response to increased N for *Cynodon dactylon* (again, however, stolon responses were far greater in response to light supply). However, they also found no decrease in stolon branching with increased N, which did not occur for *Digitaria*.

Physiological integration between ramets was not investigated here, but could be of benefit in a patchy environment, even in the absence of morphological plasticity. Caraco and Kelly (1991) discuss various ecological scenarios where physiological integration would be of benefit, and Maurer and Zedler (2002) demonstrated how physiological integration enabled a rhizomatous grass to exploit open patches.

Storage

If temporal variation in N supply occurs at a resolution longer than that relevant to nodal roots, then storage of N acquired could become an important aspect part of growth responses to N supply. In this situation, the option of 'discounting the future' must be considered (Lerdau 1992), as storage of some of the N acquired, rather than using it all for growth, could result in greater growth when N supply declines, and thus greater growth across an entire growing season. As mineralization rates are thought to decline in the ecosystems in which these species occur, it may be worth speculating that species such as *Digitaria* and *Sporobolus* do not show a large RGR response to high N supply because they allocate relatively more N acquired to storage. They may therefore show greater growth when it is measured across an entire growing season.

Measuring the N concentration of non-leaf parts would be required to test for differences in storage. In addition, as storage can involve the retranslocation of N from leaves below-ground (as has been shown for other grasses species - Heckathorn and Delucia 1996), differences in N concentration of live and dead leaf material would be informative. Unfortunately, the presence of latter storage 'mechanism' would mean that the effects of defoliation (in terms of frequency) would become a far more important factor in determining a species' 'responses to N'.

Other means of acquiring N

Arbuscular mycorrhizal fungi (AMF) have been shown to have large effect on grass growth (Alsopp 1998; Schultz *et al.* 2001), and have been found in range of southern African species, including *Urochloa mossambicensis* (Hartnett 2002). While mycorrhizal associations are more often associated with increased phosphorus uptake, Faure *et al.* (1998) report that AMF infection enhances nitrate uptake for *Lolium perenne*. Considering the growth responses of the four species in question, enhanced N uptake via AMF would only be of relevance if infection rates differ between species. In addition, if AMF infection benefits the slow-growing species more than the faster-growing ones, then it would only serve to magnify the observed differences in growth response.

The uptake of mineral N directly through leaves has been documented (Peuke *et al.* 1998), but it seems unlikely that N in this form would ever be occur in significant amounts on the canopies of grazing lawn grasses.

Interactions with other limiting factors

N is only one of many factors which can affect the growth of a plant, and it is likely that the observed pattern of growth responses would have been quite different if the effect of other resources or stresses were allowed to influence growth in a realistic way.

Defoliation

Direct effects

While the biweekly defoliation certainly had some effect on growth, the frequency (or height) of clipping may have been too lenient to simulate defoliation regimes under which the grazing lawn species evolved. Certainly, other lawn species have been grown under more severe clipping regimes (e.g. *Sporobolus ketrophyllus* - McNaughton *et al.* 1983) which may have altered growth responses in this study. It appears that *Urochloa* would have suffered the most from more intensive defoliation (**Fig. 5**), probably as a result of its morphology. This suggests that more frequent defoliation would have reduced differences in RGR, and less would have magnified them.

Indirect effects

Defoliation can affect growth indirectly as well, in that it may alter allocation patterns, even for grazing-tolerant species (Ruess *et al.* 1983). These effects are apparently result from the reduction in carbohydrate supply to roots which occurs after clipping (Dawson *et al.* 2000). As mentioned, clipping may have suppressed allocation to lateral roots at low N supply, and this effect is likely to be influenced by clipping frequency. Defoliation has also been shown to alter the vertical distribution of roots, resulting in a larger proportion of roots in upper soil layers, which in turn could increase vulnerability to water stress (Dawson *et al.* 2000).

Clipping has been shown to increase rhizodeposition (Dawson *et al.* 2000; Hamilton III and Frank 2001), which has two potential feedback effects. Firstly, it may increase the supply of other potentially limiting minerals, particularly phosphorus and zinc (Dawson *et al.* 2000). Secondly, it may increase the supply of N, by stimulating net N mineralization (this is discussed in more detail in the following chapter (Hamilton III and Frank 2001). Therefore, clipping frequency could have an important effect on the temporal variation in N supply, and this interaction would more than vary with the initial (or background) N supply from the soil. Finally, clipping has been shown to reduce mycorrhizal infection, which could lead to a short supply of other minerals, such as P (Alsopp 1998).

Light

Light has been shown to have strong interactive effects with N supply, with increased growth responses to N supply nutrient supply under high light (Meziane and Shipley 1999). *Urochloa* at least experienced a non-saturating supply of light, and may have responded differently at higher light intensities which occur in summer in HUP (over 2000 umol. m^{-2} s^{-2}). However, as low light generally only limits photosynthesis at high nutrient supply (Lambers *et al.* 1998), a higher light supply would probably only serve to increase the difference in maximum RGR seen between *Urochloa* and the other species.

Other soil resources

Minerals

The uptake of one mineral can be affected by the supply of another and, for example, nitrate uptake has been shown to be reduced by phosphorous deficiency (Lambers *et al.* 1998). However, this is not a widely tested pattern, and Cui and Caldwell (1998) found that for the grass *Agropyron desertorum*, N uptake from enriched patches was unaffected by P fertilization. A limited supply of another resource could however, affect allocation patterns, thereby affecting the growth response to N indirectly. Bloom *et al.* (1985) and Gleeson and Tilman (1992) predict that plants adjust allocation so that limitations imposed are nearly equal for all resources, and the RGR patterns observed here might have been different if the experiments had been performed with, for example, low P supply.

Water

The imposition of water stress would be particularly interesting, considering that the environments in which these species occur experience regular drying of the soil during the growing season. In the field, *Sporobolus* appears to be less affected by summer droughts - its leaves do not wilt as quickly as the other species, and partially wilted leaves appear to regenerate (pers. obs.). In addition, a closely related species (*Sporobolus stapfianus*) is a resurrection plant (Ghasempour *et al.* 2001). It is possible then that *Sporobolus* is adapted to slow yet continuous growth for a much longer portion of the growing season, while *Urochloa* is adapted to rapid growth but only at times of both high N and water supply.

Vegetative growth versus reproduction

It is difficult to argue that seed recruitment is of much significance to the reproduction of clonal species such as those in this study. However, the prolific flowering observed by these species (both in the field and in these experiments) suggests that it could be. As mentioned, germination responses have been shown to vary in response to N supply – further experimentation is required to determine if this is also the case for lawn grasses. Furthermore, taking into account the effect of seed size might also lead to a contradiction of the RGR pattern observed here. For example, Elberse and Berendse (1993) found that the Ellenberg N numbers for eight Netherlands species did not correlate with RGR, but did correlate strongly with seed weight.

Conclusion

Differences in both RGR and biomass allocation suggest that the four grazing lawn species tested in solution culture experiment do not share a similar, high demand for

N. Furthermore, the results of the sand culture experiment show that three of these species do not share a common preference for a particular source of inorganic N. Provided they give a reasonable accurate representation of growth responses under natural conditions, these results falsify the hypothesis that N supply is a key ecological process controlling the distribution of grazing lawns. However, even though the treatments applied covered an extreme range of N supply (both in terms of source and amount) which might encompass that which occurs in an grass-grazer ecosystem, this conclusion is regarded as tentative. This is because of increasing evidence that plant growth responses to a single resource are dependant on a number of complicating factors, such as spatio-temporal variation in supply rates, and the supply of other resources.

CHAPTER 3

THE EFFECTS OF NITROGEN, WATER AND LIGHT IN THE FIELD

INTRODUCTION

This chapter investigates the effects of nitrogen (N) supply on lawn grass growth under more natural conditions than in the previous chapter, and investigates the importance of two other potentially limiting resources, light and water. The previous chapter indicated that the rate of N supply is not likely to play a large role in the distribution of lawn grasses, at least for certain species, but as discussed in that chapter, the results of short-term experiments conducted under controlled conditions cannot be extrapolated to an ecosystem with much confidence. While the experiments described in this chapter are not of a much longer time-scale, they do involve more realistic environmental conditions, in that they incorporate the growth of co-occurring species (i.e. competition). Three field experiments were conducted, two at the mesic end of the HUP rainfall gradient, and one at the arid end.

The mesic extreme

The virtual absence of grazing lawns from areas of highest rainfall in HUP (receiving approximately 1000mm per year) was hypothesized to be a result of a low supply of nitrogen (N) in chapter 1. A low supply of N, or any other mineral, in these environments could be caused by the rapid growth and regrowth of bunch grasses (discussed below), or may simply reflect an abiotic limit to rates of N cycling. Alternatively, faster bunch grass regrowth could lead to significant shading of lawn grasses between grazing events, and thus the formation of a grazing lawn community. These competitive effects are depicted in **Fig. 3.1**. To test them, a factorial gradient experiment (the **mesic-end experiment**) was conducted using lawn grasses transplanted into a bunch grass community at a high-altitude, high-rainfall site in HUP. To test for possible abiotic constraints on N supply (and any other abiotic factors affecting lawn grass growth) an additional experiment (the **herbicide experiment**) was conducted. This simply involved growing lawn grasses in the absence of any competition at the same site.

Bunch grass growth and rainfall

The formation and persistence of grazing lawns lower down the HUP rainfall gradient indicates that grazing adequately reduces any bunch grass competition in these parts. This implies that it is the higher rainfall at the mesic end of the gradient which increases the competitive effect of bunch grasses. Considering the widely established positive relationship between rainfall and bunch grass growth (McNaughton 1985a; Sala *et al.* 1988; Barnes *et al.* 1991; O'Connor 1994; Ojima *et al.* 1999; Lauenroth *et al.* 2000; Knapp and Smith 2001; O'Connor *et al.* 2001), this is plausible. In addition, data collected by the ZLGP indicates that the productivity of bunch grasses increases up the rainfall gradient in HUP, for both grazed and ungrazed communities (unpublished data). The mechanisms by which this increased growth could reduce the availability of N and /or light to the point that lawn grasses are excluded are discussed below.

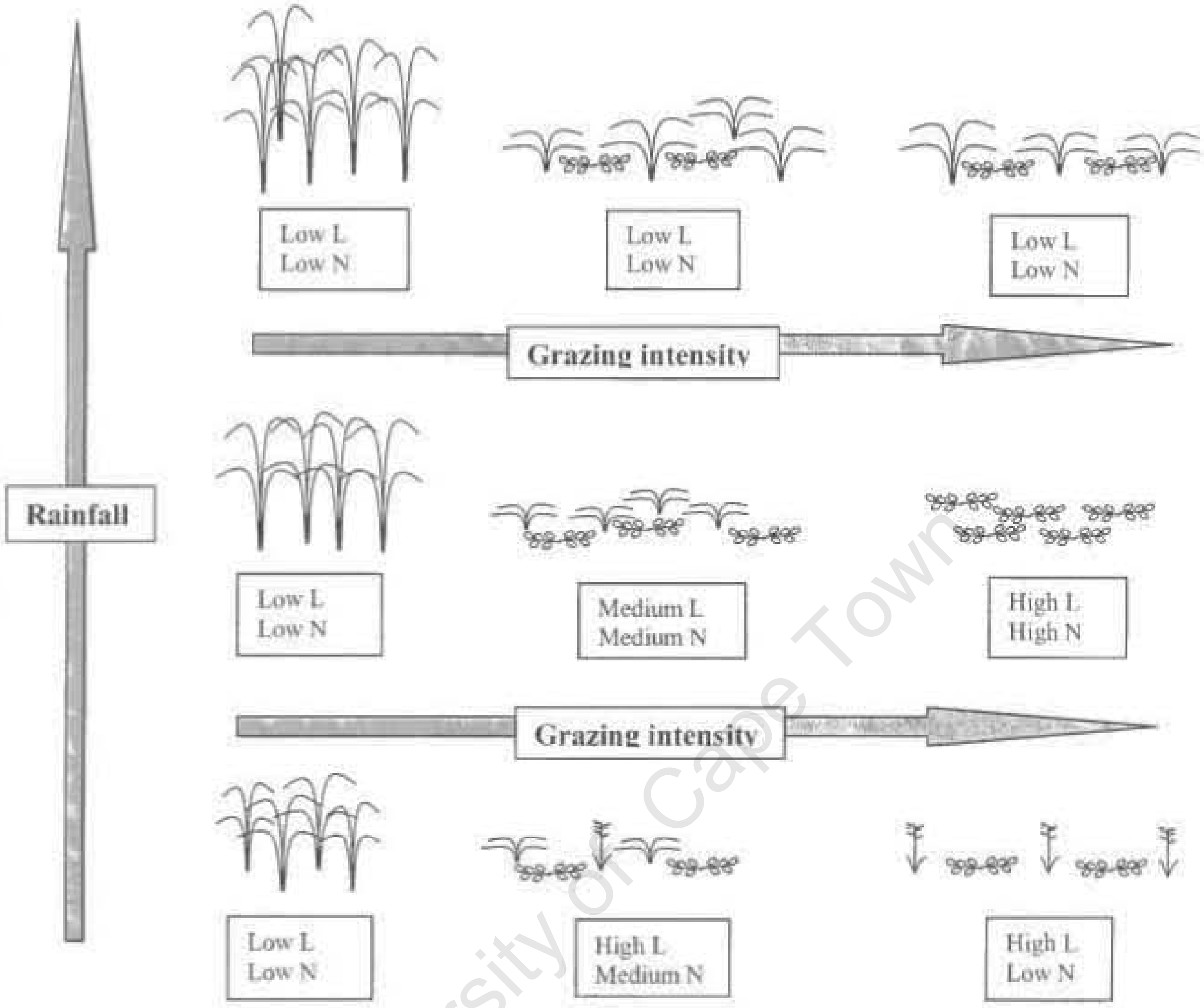


Figure 3.1. Schematic representation of the hypothesized effect of rainfall on changes in light (L) and inorganic nitrogen (N) supply that occur with increased grazing, at the edge of an existing grazing lawn or during the conversion of a bunch grassland to a grazing lawn.

= lawn grass; = bunch grass, = unpalatable grasses and forbs.

Competition for N

The results of the previous chapter indicated that the two most common lawn species in the more mesic parts of HUP, *Digitaria longiflora* and *Dactyloctenium australe* (referred to by their generic names hereafter) can grow close to their maximum relative growth rate (RGR) at low concentrations of inorganic N in solution culture. However, this does not necessarily exclude the possibility of N supply severely limiting their growth under more natural conditions. This is because:

1. The relationship between rates of N supplied in the solution culture experiments, and those that occur in the field are unknown. It is possible that N supply from the soil in the most mesic parts of HUP is as low as the lowest levels supplied in the solution culture experiment.
2. As was discussed in more detail in chapter 2, many factors could modify growth responses to N under more natural conditions (spatio-temporal variation in supply rates and low supply rates of other resources in particular).

It is possible that the enhanced bunch grass growth that occurs with greater rainfall, even with heavy grazing, leads to a significantly lower supply of N (to lawn grasses) than in drier environments where grazing lawns are common. Such a reduction could occur directly, by means of litter inputs from bunch grasses affecting soil N cycling, or indirectly, through a change in fire regime. However, the potential for heavy grazing to ameliorate such reductions also needs to be considered.

Bunch grass growth and N cycling

The idea that bunch grasses create low levels of N supply is not a new one, and has been reported for a number of species in North America (Wedin and Tilman 1990; Tilman and Wedin 1991) in a test of the resource-ratio hypothesis (Tilman 1988). Wedin (1999) explains how high nitrogen-use efficiency (i.e. low N demand) can reduce N availability. Essentially, increased growth leads to increased allocation to structural organs (presumably an adaptation to reduce light competition and self-shading). This leads to an increase in the carbon:nitrogen (C:N) ratio of plant litter, and a relatively greater supply of C than N to microbes decomposing that litter. Ultimately, this results in more of the N in the litter becoming immobilized as microbial biomass, and less being released as plant available, inorganic N. If the relationship of increased growth and plant allocation is not altered along the rainfall gradient of HUP (by changes in e.g., species composition or edaphic factors), then it is likely that N supply does decrease up this gradient.

Bunch grass growth, fire and N cycling

Both the frequency and intensity of fire is higher in the higher rainfall parts of HUP (Balfour and Howison 2002), as a result of management actions and the enhanced growth of the bunch grasses there. An increased frequency of burning can decrease the availability of N in grass-dominated systems. Data collected in HUP shows that for one of the most common bunch grasses species in the mesic areas of HUP, *Themeda triandra*, leaf blade N concentrations were lower in areas which been more frequently burnt over the past 40years (McLean 1999). Studies of the effects of fire on a North American tallgrass prairie show that annually burned sites have lower rates of N mineralization, while the grass communities on these sites have lower N concentrations and show a greater growth response to N fertilization (Turner *et al.* 1996; Blair 1997). The mechanism responsible appears to be that frequent burning leads to a relatively greater contribution of roots (which have high C:N tissue concentrations) than shoots (low C:N) to plant litter, resulting in overall increase in the C:N soil organic matter, and decreased rates of net mineralization (Blair 1997; Johnson and Matchett 2001). It has also been suggested that fires of high intensity deplete the total N pool of the soil, which could also reduce rates of N mineralization. The results of Jensen *et al.* (2001) show that high fire intensity in a mesic savanna can increase N losses from microbial biomass in the upper soil layer.

The assumed N demand of bunch grasses

If bunch grasses do create an environment with a supply of N so low that it inhibits the growth of species such as *Digitaria* and *Dactyloctenium*, then it follows that the bunch grasses themselves must tolerate this supply. There is no direct evidence to support this assumption, and the N responses of the bunch grasses species of HUP are largely unknown. However, the responses of the ubiquitous *Themeda triandra* to N fertilization offers some support. At a grassland site with slightly lower rainfall than the mesic part of HUP, fertilization with ammonium nitrate or limed ammonium nitrate led to large reduction in the abundance of this species (le Roux and Mentis 1986). At the same time species common on heavily grazed communities increased in abundance, suggesting that it is a tolerance of a low supply of N which enables *T. triandra* to dominate over these species. Similar results have been found for fertilizer experiments in the more arid, Highveld grasslands of South Africa (O'Connor and Bredenkamp 1997; Snyman 2002) and in North American tallgrass prairie (Tilman 1990; Wilson and Tilman 1991; Wedin and Tilman 1993).

Grazing and potential increases in N supply

For bunch grass growth under mesic conditions to reduce N supply to a critically low level, this effect would have to persist in the presence of heavy grazing for lawn grasses to be excluded. If heavy grazing were to increase the rate of N supply to levels as high as those found on grazing lawns in drier areas of HUP, then the hypothesis of competition exclusion and N must be false. There is as yet no evidence available to determine the extent to which grazing affects rates of mineralization in the most mesic parts of HUP. However, the little evidence found regarding the effects of grazing on soil processes in mesic areas suggests that, for a given level of bunch grass growth, increases in N supply created by grazing could be lower with greater rainfall. This is because of denitrification, the process whereby mineral N in the soil is converted to gaseous N and lost to the atmosphere. Frank and Groffman (1998a) found that denitrification increased substantially with grazing in Yellowstone National Park, but only at sites with the highest soil moisture, and Groffman *et al.* (1993) report that temporal variation in denitrification (on a monthly basis) was related to soil moisture for mesic, tallgrass prairie. If the same pattern occurs in HUP, then an incipient grazing lawn at the mesic end of the rainfall gradient may not only 'start' with lower mineralization rates, but also lose any increased supply of mineral N to denitrification.

Competition for light

Shading is an obvious part of bunch grass competition in the absence of grazing, but enhanced bunch grass growth with greater rainfall could result in the shading of lawn grasses on an incipient lawn as well. In other words, greater rainfall may reduce the rate at which grazing reduces shading. This could occur in two ways:

1. Directly, in that in the interval between grazing events, bunch grass regrowth could be fast enough to begin shading lawn grasses (while in drier areas it would not).
2. Indirectly, through the effect of enhanced growth increasing burning frequency. As burned areas are highly attractive to grazers (Vinton *et al.* 1993; Lutge 1995; van de Vijver *et al.* 1999), burning could reduce the frequency with which grazers visit incipient grazing lawns, and allow for greater regrowth of the grazed bunch grasses there.

Thus the establishment of lawns at the mesic end of the rainfall gradient in HUP may be contingent upon the ability of lawn grasses to endure regular episodes of shading.

N and light interactions

At the leaf level, a strong positive relationship for leaf N concentration and the maximum (light saturated) rate of photosynthesis, for a wide range of species (Lambers *et al.* 1998). The relationship between leaf N concentration and quantum-use efficiency (the rate at which photosynthesis increases with light supply) is, however, rather weak (Gastal and Durand 2000). Therefore, if leaf level responses control plant level responses, one would expected an additive effect of N and light supply on plant growth, i.e. lawn grasses should not respond to increased N supply unless light supply is maximal. However, N supply affects physiological processes other than photosynthesis, and it is possible that an interactive effect would emerge at the plant level.

In order to test for the effects of light and N supply, as well as any interaction between the two, a fully factorial experimental design was used for the **mesic-end experiment**, with N and light 'addition' treatments applied at a gradient of levels. Ideally, the experiment would have been done on an incipient grazing lawn at the mesic end of the rainfall gradient, using established lawn grasses. However, the scarcity of such sites made this impossible. Instead two species of lawn grasses were transplanted from lawns further down the rainfall gradient into a lightly-grazed bunch grass community. A major disadvantage of this approach was that it required simulation of light and N supplies that would occur with heavy grazing. It did bring the advantage, however, that the control treatments represented a null hypothesis of the overall hypothesis, i.e. if lawn grasses do not suffer N or light shortages when grown with ungrazed bunch grasses, then the supply of these resource could not possibly be limiting when grazing intensity is increased.

The arid extreme

In semi-arid areas of HUP, mean annual rainfall falls to below 650mm. While grazing lawns are common at this end of the rainfall gradient, it appears that lawn grasses cannot persist in areas that experience the most intense grazing (e.g. around wallows and water points). Relative to grazing lawns, these areas are characterized by a low basal cover of herbaceous species with unpalatable grass species and forbs more common than lawn species (chapter 1). While not often described in the literature this pattern, of heavy grazing resulting in mostly bare soil, is common on rangelands and in game reserves in the semi-arid parts of South Africa (pers. obs.). One example of a formal description comes from a cattle-grazed, semi-arid highveld grassland (Fuls 1992). Fuls measured the relative abundance of species along a grazing gradient, and reports that tufted perennials were replaced by 'creeping grasses' which then disappeared at the highest intensity of grazing (resulting in a basal cover as low as 1%). In addition, grazing-tolerant species have been reported to decline in heavily-grazed, semi-arid environments during dry years (O'Connor 1991a). It therefore it appears that the transition from a mesic to semi-arid climate marks the boundary at which grazing tolerant grasses can persist under conditions of heavy grazing.

Unlike the mesic extreme, competition from bunch grasses does not appear to play a role in the absence of grazing lawns at the arid extreme, as these species are relatively far less abundant than in the heavily-grazed areas higher up the rainfall gradient (pers. obs.) Instead, the exclusion of lawn grasses from the arid extreme appears to be a direct result of abiotic factors. Specifically, a shortage of N and / or water are hypothesized to be the factors responsible (**Fig. 4.1**). In other words, it is proposed that a reduction in rainfall reduces the supply of water and / or N, which reduces the intensity of grazing which lawn grasses can endure. This assumes that grazing intensity is no higher at the arid extreme of HUP. While there is no data to support this assumption, the distribution of watering points in the park, as well as personal observations, suggest that it is true. To test for the potentially limiting effects of water and N supply an experiment (the **arid-end experiment**) was conducted at one of the most arid grazing lawns in HUP.

Why water?

While low rainfall is clearly associated with the absence of lawns, both in the arid parts of HUP and other parts of southern Africa, almost no studies were found which directly measure the effects of water supply on grazing-tolerant grasses. One exception is the irrigation of a grazing lawn in the Serengeti ecosystem, which did increase above-ground peak biomass significantly in the presence of grazing (Williams *et al.* 1998). As water plays a large role in all soil processes, it is likely that a reduced supply of soil water results in a reduced supply of other soil resources as well. Of these, N is considered the most important.

Why N?

The growth response of *Urochloa* in solution culture (chapter 2) suggests reductions in N supply created by reduced rainfall need not be particularly large to exclude this common, semi-arid lawn grass species. The idea that N limits grass growth in semi-arid savannas has been suggested before (McNaughton *et al.* 1988) based on growth responses of grazing lawns in the Serengeti to rainfall events at different times in the growing season, and on fertilization experiments in the Sahel.

Low rainfall may reduce N supply for three reasons.

1. Rates of N mineralization are affected by soil moisture and have been shown to decline at low soil water content (Wiltshire 1990; Mazzarino *et al.* 1998; Tracy and Frank 1998).
2. If reduced rainfall reduces plant growth, then this will feedback into reduced litter inputs (regardless of grazing intensity), and correlations between vegetation cover and soil organic matter have been reported for semi-arid ecosystems (Evans and Ehleringer 1994; Vinton and Burke 1995; Briske and Derner 1998; Mazzarino *et al.* 1998). The amount of soil organic matter in turn correlates strongly with rates of N mineralization, and with flushes of inorganic N that occur subsequent to the death of soil microbes, which is likely to be an important source of N in semi-arid systems (Mazzarino *et al.* 1998),
3. N uptake is contingent on water uptake, as at least part of the uptake process involves mass flow (Lambers *et al.* 1998). Diffusion rates of inorganic N (and all other minerals) also slow as soil dries (Lambers *et al.* 1998).

N and water interactions

The effects of interactions between N and water supply on grass growth are not well understood, even for pastoral systems (Gastal and Durand 2000). However, there is some evidence that the ability of a plant to acquire N (in terms of specific uptake rate) is reduced by water stress. In a split-root experiment Buljovcic and Engels (2001) found that individual roots of *Zea mays* had lower N uptake when whole plants were water-stressed. Likewise, there is some evidence that N supply affects a plant's ability to tolerate water stress. Volaire *et al.* (1998) showed that survival of two C_3 agricultural species during growing season droughts was enhanced by, amongst other traits, rapid N uptake after rewatering. Based on this, one would expect an interactive effect of water and N supply.

Again a fully factorial, gradient design was chosen so that potential interactions could be identified for the **arid-end experiment**. Ideally treatments would have involved reductions of water and N availability, with a decline in lawn grass growth as the expected response. However, it proved to be cheaper and more practical to increase, rather than decrease, the supply of these resources. Treatments therefore involved irrigation and fertilization, and it was hoped that (together with a predicted drought) lawn grass populations would show either greater growth, or smaller reductions in growth, with increasing treatment levels.

To summarize, three field experiments were performed, all over the same growing season. The **herbicide experiment** tested for abiotic constraints on the growth of lawn grasses at the mesic end of the HUP rainfall gradient. The **mesic-end experiment** tested the hypothesis that a shortage of light, N or both, created by the growth of bunch grasses, excludes lawn grasses from the most mesic parts of HUP. The **arid-end experiment** tests the hypothesis that a shortage of water, N or both excludes lawn grasses from the driest parts of HUP.

METHODS

Herbicide experiment – abiotic constraints

Site

The experiment was conducted on the crest of a hill, at an altitude of 500-510m above sea level ('Hilltop' in **Fig. 1.1**). The bedrock is dolerite (Research 1979) and the soils of the Hutton form (Barnard and Barow 1984). Total soil N and P were measured as 5.41 mg g^{-1} (SD 0.56) and 650:g g^{-1} (SD 37.5) respectively (ZLGP, unpublished data). Mean annual rainfall, recorded at the nearby Hluhluwe Research Centre was 997mm (1980 to 2002). Dominant grass species (all tall bunch grasses) are *Hyparrhenia filipendula, Themeda triandra, Setaria sphacelata* var. *sericea*, *Eulalia villosa* and *Sporobolus africanus*. Woody species consist of forbs and a few shrubs (gullivers) only. Grazing pressure is light, although the area is regularly visited by zebra and buffalo (pers. obs.). The area is burned almost every year, in late winter or early spring (D. Balfour, pers. comm.) and was burned on the 13 Sept. 2000, as part of a large (100's of hectare) management burn.

Experimental design

The experiment consisted of a single block with a single treatment (herbicide versus control), and four replicates for each treatment. A single 8 x 8 m^2 plot was marked out and divided into sixteen 2 x 2 m^2 subplots, with each assigned as either control or herbicide treatment, in a checker-board fashion. In the herbicide subplots, all grasses emerging a few weeks after the burn were sprayed with a herbicide (Round-up). Any plants which had survived this, or which invaded the treatment subplots subsequently, were removed by hand. On the 13th and 14th October 2000, approximately two weeks after the herbicide application, individual plants of *Digitaria longiflora* and *Dactyloctenium australe* (*Digitaria* and *Dactyloctenium* hereafter) were transplanted into the plot (2 individuals per species per subplot). This involved digging a small hole with a pick (approximately 5cm in diameter and 10cm deep), inserting transplants and replacing and compacting dug soil. Transplants were watered on the same day, and substantial rainfall occurred the night after transplanting as well. One tuft of each species was transplanted into each corner of the central 1 x 1 m^2 of each subplot, with two tufts per species per subplot. The transplants were dug up from grazing lawns elsewhere in the park (*Digitaria* all from one location, *Dactyloctenium* from two).

Measurements

Plants were harvested near the end of the growing season, from the 1st to the 4th May 2001. Biomass was estimated differently for the control and treatment due to the large differences in plant size. For the control, the above-ground (AG) parts of each plant were removed by cutting through the nodal roots slightly below the crown. The number of nodal roots per plant were then counted before removing the remaining upper parts of these roots. AG parts (including crowns) were then separated in live and standing dead material and dried at 70°C for 3 to 4 days.

The large size and spread of the plants meant that this method was not practical for the herbicided subplots. Instead the cover of each species in these subplots was estimated by counting the number of 10 x 10cm cells in which they occurred using a sub-divided quadrat. All *Digitaria* and *Dactyloctenium* live material in one or two of the 10 x 10cm cells (per subplot) was harvested in the same manner as the control plants in order to estimate average AG biomass on an area basis. In addition, many stolons grew beyond the 2 x 2 m^2 area of their subplots and penetrated the surrounding bunch grass. These 'escaped' stolons were harvested in the same manner as the control transplants.

Flower production was measured during the second half of the growing season. In each subplot, the total number of live and standing dead (i.e. brown) racemes were recorded for each species. Flowering culms which had lost their racemes to grazing were counted as two racemes (which is the usual number of racemes on a flowering culm for these species – pers. obs.). Racemes were counted nine times through the growing season (including a sample at harvest) starting in early December 2000. The interval between consecutive sampling dates was between 7 and 29 days.

Response variables

Biomass

In order to estimate whole plant biomass of the plants harvested, root biomass was estimated from the ratio 'number of nodal roots / AG biomass'. Non-linear regressions of root mass fraction (RMF) against 'number of nodal roots / AG biomass' were obtained from the data collected from plants grown in solution culture (chapter 2). As RMF must be between 0 and 1, asymptotic non-linear functions were used. For *Digitaria*, a non-rectangular hyperbola provided the best fit:

$$\mathbf{RMF} = (0.54 \times \mathbf{NOD}) / (15.25 + \mathbf{NOD});\ n = 37;\ r^2 = 0.76;\ p < 0.001$$

For *Dactyloctenium*, a monomolecular function provided the best fit:

$$\mathbf{RMF} = 0.64(1 - e^{-0.021 \times \mathbf{NOD}});\ n = 37;\ r^2 = 0.45;\ p = 0.001$$

(NOD refers to the ratio of 'number of nodal roots / AG biomass').

The range of the data for 'number of nodal roots / AG biomass' was greater for plants harvested in this experiment than for those used to estimate the regression. However, it was only the maximum values that were greater (approximately 150 for harvested plants and 100 for solution culture plants) and as both functions are asymptotic, the error at this end of the relationship is not likely to be large. Whole plant dry weight was then calculated from the estimates of RMF and the AG dry weights. For the herbicide treatments, the RMF of the escaped stolons was assumed to be the same as the mean of the control transplants. The resulting estimate of total dry weight for these parts was added to that calculated for transplants within the boundaries of the subplots, to produce a total for each species in each subplot (the percentage of the total made up of 'escapee stolons' varied between 0 and 5.5 %).

Raceme production

Raceme production in the interval between two consecutive sampling dates was calculated as the number of live and dead racemes (in a subplot) at one sampling date minus the number of live racemes in the previous sample. If there were fewer racemes at the second sample, production was set to zero. This was done separately for each species. This method incorporates the following assumptions:

1. a live raceme at one sampling date would still be present at the next (either alive or dead),
2. if a raceme was produced between a pair of samples, it will be present at the second sampling date (either alive or dead)
3. dead racemes present at one sampling date would not persist to the next one.

Based on observations in the field, these assumptions are reasonable. Raceme production for each interval was then summed to produce a total for the entire sampling period.

Statistics

The relative growth rate of the 'population' of transplants in each subplot could not be determined as only one harvest was performed. Therefore, growth was analysed in terms of final biomass only, and it was simply assumed that:

1. initial biomass did not differ significantly between treatments and controls;
2. biomass accumulation was either linear or followed a similar pattern (in terms of rates of change) for treatments and controls.

Final biomass proved to be orders of magnitude greater on the herbicide treatment and no transformation of the data produce equal variance between control and herbicide treatment. Therefore differences were tested with the non-parametric Mann-Whitney U test (using a separate test for each species). RMF and raceme production data were analysed in the same way (although treatment differences were not as large, homogeneity of variance could still not be achieved with transformation).

Mesic-end experiment - the effects of N and light

Site

The same site was used as for the **herbicide experiment**, and the plots created for this experiment were within a few hundred metres of the one used for that experiment. A few shrubs growing in one of the plots were cut down at the start of the experiment and re-cut when necessary.

Experimental design

The experiment consisted of three blocks, each containing one replicate of 5 levels (including one control) of two gradient treatments. Each block consisted of a 12 x 12 m^2 plot. Plots were chosen on the basis of the homogeneity of the vegetation, an absence of trees, depressions and termitaria, and similarity of slope. Each plot was divided into 5 rows and strips of 2m width, as well as boundary rows and strips of 1m width, creating twenty-five 2 x 2 m^2 subplots within a boundary layer 1m wide (**Fig 3.2**). The two gradient treatments were orthogonal, with levels increasing down the strips of a plot for one treatment, and down the rows for the other. This resulted in a single control subplot (receiving control level for both treatments), with the remaining 24 subplots receiving one of the 24 potential combinations of the 4 levels of the each treatment).

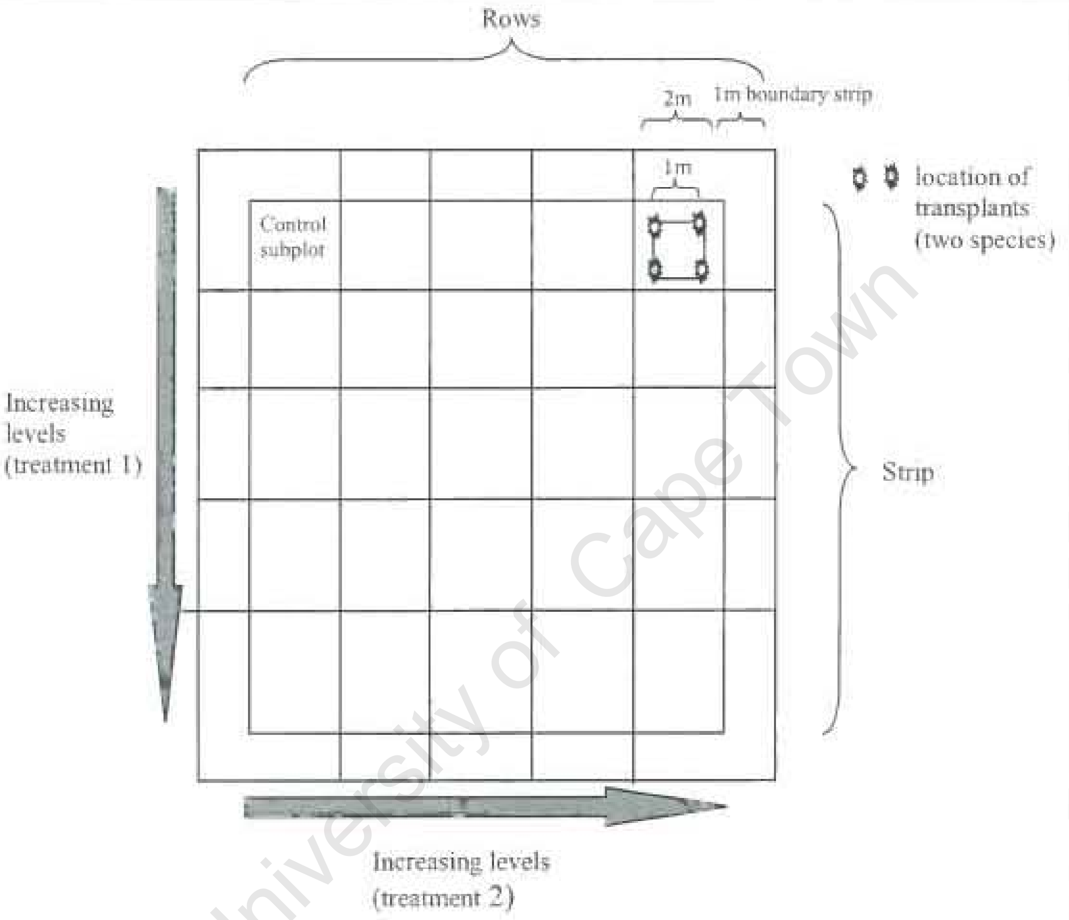


Figure 3.2. The layout of the plots used for applying the two orthogonal treatment gradients in the mesic-end and arid-end experiments conducted in Hluhluwe-Umfolozi Park.

Plant material

Transplanting was done as for the **herbicide experiment**, except that the first transplants were planted on the 14th September 2000, and those which died (20 of the original 300) were replaced on the 13th and 14th October 2000.

Treatments

N addition

The N addition treatment consisted of bimonthly fertilization. An ammonium nitrate solution was sprayed onto subplots with fire-fighting backpacks (**Fig 3.3A**). See **Table 3.1** for treatment levels. Concentrations of the solutions applied ranged from approximately 16mM to 95mM N. The NH_4NO_3 was applied three times through the growing season, on the 15th or 18th October 2001, 6th December 2001 and 30th or 31st January 2002. Each application was done within a day since the last rains and soils surfaces were wet at application. As there was a slight slope at this site, plots were arranged so that the N addition gradient ran down slope, with the highest treatment level at the bottom end of each plot.

The resulting total amount of N added was far less than in other fertilizer experiments - increased grass growth in planted pastures or intensely grazed rangelands has been achieved using levels between 70 and 400 kg N ha-1 year-1 (Grunow and Rabie 1985; le Roux and Mentis 1986; Barnes *et al.* 1987; Andren *et al.* 1996; Jarvis 2000), while responses of natural or non-crop monoculture communities are typically reported to occur in the range of 54 to 480 kg ha^{-1} $year^{-1}$ (Day and Deitling 1990; Bobbink 1991; Tilman and Wedin 1991; Wilson and Tilman 1991, 1993; Turner *et al.* 1996; Foster 1998). While adding such large amounts of N would be more likely to produce measurable growth responses, such responses would not necessarily be ecologically informative in this experiment, as it is unlikely that grazing could increase N supply to these levels. Furthermore, the large growth increases reported for the experiments cited above suggest that measurable responses would occur below the levels used. Snyman (2002) for example, found significant changes in basal cover and AG production for a community of C_4 grasses receiving only 10 kg N ha^{-1} $year^{-1}$.

Light addition

The second treatment was designed to increase the amount of light reaching the transplanted lawn grasses below the canopy of bunch grasses amongst which they were planted. While light 'addition' has been achieved by the use of mirrors (Eek and Zobel 1997) this is not feasible in an environment which contains many large herbivores, or where bunch grass canopies can be extremely dense. Instead light addition was attempted by tying the canopies of bunch grass foliage together, as the bunch grew taller through the growing season (**Fig. 3.3B**). In order to create a gradient of light 'addition', different proportions of the bunch grasses shading a given transplant were selected to be tied up (**Table 3.1**).

Figure 3.3. Addition of nitrogen, light and water to lawn grasses for the mesic-end and arid-end experiments conducted in Hluhluwe-Umfolozi Park, South Africa (December 2000). **A**. NH_4NO_3 was sprayed onto bunch grassses and tranplants of *Digitaria longiflora* and *Dactyloctenium australe* (Hilltop site). **B**. 'Light addition' by means of tying up canopies of bunch grasses surrounding the lawn grasses transplanted at the Hilltop site (the arrow shows a *D. longiflora* transplant). **C**. Irrigation of a grazing lawn dominated by *Urochloa mossambicensis* at the Mbuzane site.

To quantify differences in light penetration created in this way, photosynthetically active radiation (PAR) was measured using a Sunfleck PAR Ceptometer (Decagon Devices, Inc.). Measurements were made in early December, once the bunch grass canopy had developed enough to begin shading the transplants, and in late January and early April, to capture shading effects as canopies grew to full size. Measurements were performed on an overcast day, to eliminate the problem of a directional light source that occurs on a clear day (Sunfleck PAR Ceptometer Operators Manual, Decagon Devices, Inc.). An integrated measurement was taken above the canopy, as well as 10 to 25 point measurements directly above the 'canopy' of each lawn grass below. In this way the proportion of PAR penetrating to each transplant could be calculated. This measure was then averaged for the pair of transplants of each species in each subplot, and then averaged across the three sampling dates. The resulting values were used as a continuous variable in the analyses. Control subplots had a PAR transmission of 63% (standard deviation = 16%).

Due to the activity of large herbivores at the site, the strings used to tie the bunch grasses were frequently pulled off or eaten. While strings were checked and replaced twice a week for most weeks, logistical problems prevented this being done every week. As a result, the light addition treatment was not applied evenly throughout the growing season, and many transplants on the higher light addition levels experienced an erratic mix of shading and full sunlight.

Measurements

Morphological measurements were made on the transplants on the 18th and 19th October, approximately one month after transplanting. A few transplants had died prior to this date, and these were replaced shortly afterwards and measured a month thereafter, on the 1st December. For each transplant, the two longest orthogonal axes of the crown(s), and the length of all culms (shoots and stolons) were measured and the number of green leaves counted. In addition, at the time of transplanting, an additional 20 *Digitaria* and 11 *Dactyloctenium* plants were measured in the same manner, cleaned of soil (using water) and dried at 60°C for 3 to 4 days. These were then used to determine an empirical relationship between the morphological variables measured and the dry weight of the transplants. Plants were harvested from the 12th to 26th April 2001, in the same manner as the control plants in experiment 1. Raceme production was measured in the same way as in experiment 1.

Response variables

Initial (whole plant) biomass was estimated from least squares, non-linear regressions on the morphological variables obtained from the subsamples harvested at the time of transplanting.

For *Digitaria*: $\textbf{dry weight} = 0.0555(\textbf{tuft area})^{0.808} + 0.0483(\textbf{total culm length})^{0.652}$

$r^2 = 0.89$; $n = 20$; $p < 0.001$.

For *Dactyloctenium*: $\textbf{dry weight} = 0.00146(\textbf{total culm length})^{1.69}$

$r^2 = 0.80$; $n = 11$; $p < 0.001$.

Final biomass could not be calculated in this manner, as when subsamples were dug up at harvest, it was observed that over 90% of nodal roots had broken, leaving an unknown quantity of the distal part of the root system in the soil (this was not a problem for the initial subsample, as a similar amount of root material would have been lost for both the subsample and the transplants). Final biomass was therefore estimated in the same manner as for the control plants in the **herbicide experiment.** Raceme production was calculated in the same manner as in experiment 1.

Statistics

The fitting of a growth rate function was precluded as only two estimates of plant biomass were made and a constant rate of change of growth through the season could not be assumed. Treatment effects were therefore analysed in terms of changes in final biomass, as in experiment 1. However, the estimate of initial biomass was included in the analyses, making the assumption of equal initial biomass was not necessary.

A GLM ANCOVA was used to test for differences in final biomass, raceme production and RMF (using a separate analysis for each species). For each analysis a full factorial model was initially used, with plot as a random categorical variable and light level (proportion PAR transmission), N level (total amount of N added) and initial biomass as covariates. Each model was then simplified in a stepwise manner to obtain the smallest model which explained a similar amount of variance as the original model.

Prior to performing the GLM analyses, data were checked for normality using histograms and the Shapiro-Wilks test (StatSoft 2001). PAR transmission was skewed to the left for *Digitaria* and was therefore arcsine transformed (where $x = \text{arcsine } x^{0.5}$) – this improved normality for

Dactyloctenium as well. Initial and final biomass and raceme production were skewed to the right (for both species). Root transformation corrected the initial biomass data and root-root transformation the final biomass data. For raceme production, root transformation produced normality for the *Digitaria* data, and produced a distribution closest to normal of all standard transformations for the *Dactyloctenium* data. RMF data did not require transformation. The validity of the regression models were assessed on the basis of the F-statistic and the distribution of the residuals, and homogeneity of variance between groups was tested used Levene's ANOVA test (StatSoft 2001).

Table 3.1. Treatment levels for the 'light addition' and N addition treatments of the **mesic-end experiment**, and the N addition and water addition (irrigation) treatments of the **arid-end experiments** conducted in Hluluwe-Umfolozi Park.

Level	**Light**	**Nitrogen addition**			**Irrigation**		
	% of neighbouring bunch grass canopies tied up	Per application (mg N m^{-2})	Equivalent per year (kg N ha^{-1})		Frequency of watering (days since last rain / watering)	Resulting range in inter-rainfall intervals	Resulting mean inter-rainfall interval (SD) for ln transformed data
			Mesic-end	Arid-end			
control	0	0	0	0	-	1 – 36	1.43 (0.90)
1	25%	80	2.4	2.8	5	1 – 19	1.44 (0.85)
2	50%	160	4.8	5.6	7	1 – 19	1.41 (0.82)
3	75%	240	7.2	8.4	10	1 – 18	1.24 (0.84)
4	100%	320	9.6	11.2	14	1 – 18	1.12 (0.70)

Arid-end experiment – the effect of N and water

Site

The site chosen was less than 200m from the 'Moina' grazing exclosure of the Zululand Grasslands Project (Mbuzane in **Fig 1.1**). It is within a few hundred metres of the Black Umfolozi River at an altitude of 160-180m above sea level. The bedrock is dolerite (Research 1979) and the soils of the Shortlands form (Research 1980). Mean annual rainfall for a nearby ranger outpost (Mbuzane), approximately 50m higher, is 657mm (1981-2002). The area is generally heavily grazed during the growing season (D. Balfour, pers. comm.), and at the end of the growing season prior to this experiment, the vast majority of grass tufts had been grazed down to a few centimetres. *Urochloa mossambicensis* (*Urochloa* hereafter) is by far the most abundant species, with other common species being *Panicum maximum, Panicum coloratum,* and *Chloris virgata*. There was no tree cover within the boundaries of the plots.

Experimental design

The design was essentially the same as that used in experiment two, with two important differences:

1. The two treatments applied were N addition and irrigation (rather than light addition).
2. Time limitations prevented sampling in three plots. While two of the plots were laid out in the same manner as in experiment 2, the third was reduced to 2 rows and 5 strips. As a result, the N addition treatment was reduced to two levels (control and level 4) for this plot.

A few small shrubs (gullivers) and large woody forbs were cut down in two of the plots, and recut when necessary.

Plant material

Plant growth was measured on lawn species present at the site – no transplanting was involved. Two species were measured, *Urochloa mossambicensis* and *Panicum coloratum,* both of which are common on grazing lawns in the semi-arid parts of HUP (pers. obs.). All individuals inside the central 1 x 1 m^2 of each subplot were sampled.

Treatments

Nitrogen addition

This was done in the same manner as in the **mesic-end experiment.** However, as this experiment began earlier, additional fertilization was performed early in the season, but with only half the amount of NH_4NO_3. The dates of fertilization were the 20th September (half dose), 16 and 17th October, 10th December 2000 and 31st January 2001. Due to the lower frequency of rainfall events at this site, fertilizer was applied to dry soil on two of four occasions.

Irrigation

A gradient of five watering levels was used with one being the control (no irrigation). In order to determine appropriate watering intervals and amounts, daily rainfall records from a ranger station within 1km of the site, for 10 growing seasons (1988/89 to 1999/2000, excluding 1996/97 and 1997/98) were analyzed. 'Growing season' was deemed to be the period from the

1st September to 30th April. This data was lumped to determine the average rainfall size and inter-rainfall interval (**Table 3.2**).

Table 3.2. Summary of rainfall data recorded at the Mbuzane Outpost (Hluhluwe-Umfolozi Park) for 10 growing seasons (September to April) from 1988 to 2000. Data for both variables showed a gamma distribution - geometric means are therefore reported.

	Range	Geometric mean
Rainfall size (mm)	0.2 – 124	7.33
Inter-rainfall interval (days)	1 – 70	2.96

The effect of various watering intervals on the average inter-rainfall interval was estimated by manipulating the lumped data accordingly. Watering intervals were then selected so as to create a geometric gradient, with average inter-rainfall intervals intended to be 25%, 50%, 75% and 100% less than that of the control. However logistical problems placed a limit on the frequency with which the plots could be watered and a 100% reduction in average inter-rainfall interval was not viable. A 5-day limit on maximum inter-rainfall interval was the best that could be achieved for the most frequently watered treatment level, and the watering intervals for the remaining levels were adjusted to provide average inter-rainfall intervals of 25%, 50% and 75% of this level. Further logistical problems resulted in no irrigation being carried out for almost a month during the middle of the growing season, which distorted the intended treatment levels further.

Each watering event involved spraying each subplot with approximately 26 litres using a fire-hose (**Fig. 3.3C**) – this is the equivalent of 13mm of rainfall, which is well above the estimated average rainfall size (**Table 3.2**). The first rains at the site occurred on the 18th September 2000 and watering began on the 28th September. During the season, rainfall was recorded either from a rain gauge at the nearby ranger outpost, or from a rain gauge at a the 'Moina' grazing exclosure. The actual average inter-rainfall intervals produced by the treatments are shown in **Table 3.1** (as with the 10 year rainfall data set, the 2000/01 growing season daily rainfall was non-normal and skewed right - data were therefore ln transformed prior to calculating means).

Measurements

From the 11th - 22nd September 2001 (before spring growth had begun) the following morphometric traits were measured: the two longest orthogonal axes of each crown (including rooted stolon nodes), the length of all live culms (shoots and stolons; brown culms were considered alive if still flexible), and the number of green leaves counted. As no new growth was present, it was not obvious which plants were still alive and which died during the dry season. However, due to the unusually high rainfall of the previous season, most plants still had either green stolons, green leaves or both and vitality was judged on the presence of green tissues. In addition a subsample of 20 (*P. coloratum*) and 29 (*Urochloa*) plants were dug from the boundary area of each plot. These were measured as above, washed of soil, dried at 60°C for 3 to 4 days and weighed.

At the end of the season, from the 8th to 10th May 2001, morphological measurements were repeated, but limited to measuring the longest two orthogonal axes of the crowns.

In order to assess whether uneven grazing confounded the treatment effects, the proportion of green leaves bitten was measured. This was done on the central *Urochloa* plant in each subplot, by counting the total number of green leaves on the plant and the number of these which had been bitten. Grazed stolons were not common and were simply counted as one bitten leaf. Sampling was done on four occasions during the 2000/2001 growing season (mid-October, mid December, late January and late April).

Response variables

As with the subsamples dug up for the **mesic-end experiment**, it was observed that a large amount of root material was left behind in the soil. Therefore, initial biomass could not be estimated from morphological variables. Instead growth was estimated as a change in the total crown area of each species in each plot. For each individual crown measured, crown area was calculated as the area of an ellipse (area = $\pi.a.b$, where a and b are the two orthogonal axes). Crown areas were then summed to produce a total for the central square metre of each subplot. Due to the small number of *P. coloratum* plants, as well as the absence of this species from many subplots, only *Urochloa* data was analysed.

The proportion of leaves bitten was simply the ratio of bitten leaves to total leaves per plant.

Statistics

The reduction of one of the plots in this experiments created an unbalanced design, with some treatment combinations replicated twice and some three times. While the inclusion of plot as a blocking effect is still possible in this case, with the use of a GLM, this could produce bias against the smaller plot. Block effects were therefore omitted from the analysis (although their inclusion had no qualitative effect on the results).

To test for changes in the proportion of leaves bitten, a repeated measures regression design was required, as this variable was measured on a number of occasions in each subplot. This was done by using a GLM ANCOVA model with bitten leaves as the dependent variable, and 'date of sampling' as a random effect. The two treatments, N addition and irrigation were the covariates, and a model that includes all interactions with covariates was selected. The 'proportion of leaves' data was first ln transformed to meet the assumption of normality.

To test for differences in growth, a GLM factorial regression model was performed with final total crown area as the dependent variable and initial total crown area, N addition and average inter-rainfall interval as the independent variables. As with experiment 2, the model was simplified as necessary. The validity of both the above GLM models was assessed in the same was as for the **mesic-end experiment.**

RESULTS

Herbicide experiment

While transplants in the control treatments were able to grow under the canopy of bunch grasses, and survived until the end of the growing season, the removal of bunch grasses in the herbicided subplots produced a dramatic increase in growth. Both *Digitaria* and *Dactyloctenium* spread beyond the $4m^2$ subplots and penetrated the surrounding matrix of bunch grasses, although by the end of the growing season *Digitaria* appeared to have obtained a greater cover within the subplots. Differences in biomass were orders of magnitude greater **(Fig. 3.4A)** and significant for both species (MannWhitney U test for *Digitaria*: $U < 0.01$, $p < 0.001$; for *Dactyloctenium*: $U < 0.01$; $p < 0.001$). Furthermore, it was observed that transplants in the herbicide treatment were grazed almost every time buffalo or zebra visited the site, while transplants in the control site, which were much less conspicuous amongst the bunch grasses, were hardly ever grazed. Therefore differences in final biomass are likely to underestimate differences in growth.

While differences in RMF were not significantly different between treatments, both species showed more allocation to roots in the control treatments **(Fig. 3.4B)**.

Raceme production was significantly higher for *Digitaria* (Mann Whitney U test: $U < 0.01$, $p < 0.001$) but not for *Dactyloctenium* – this was most likely because of an anomalous lack of raceme production by this species in one of the herbicide subplots **(Fig 3.4C)**.

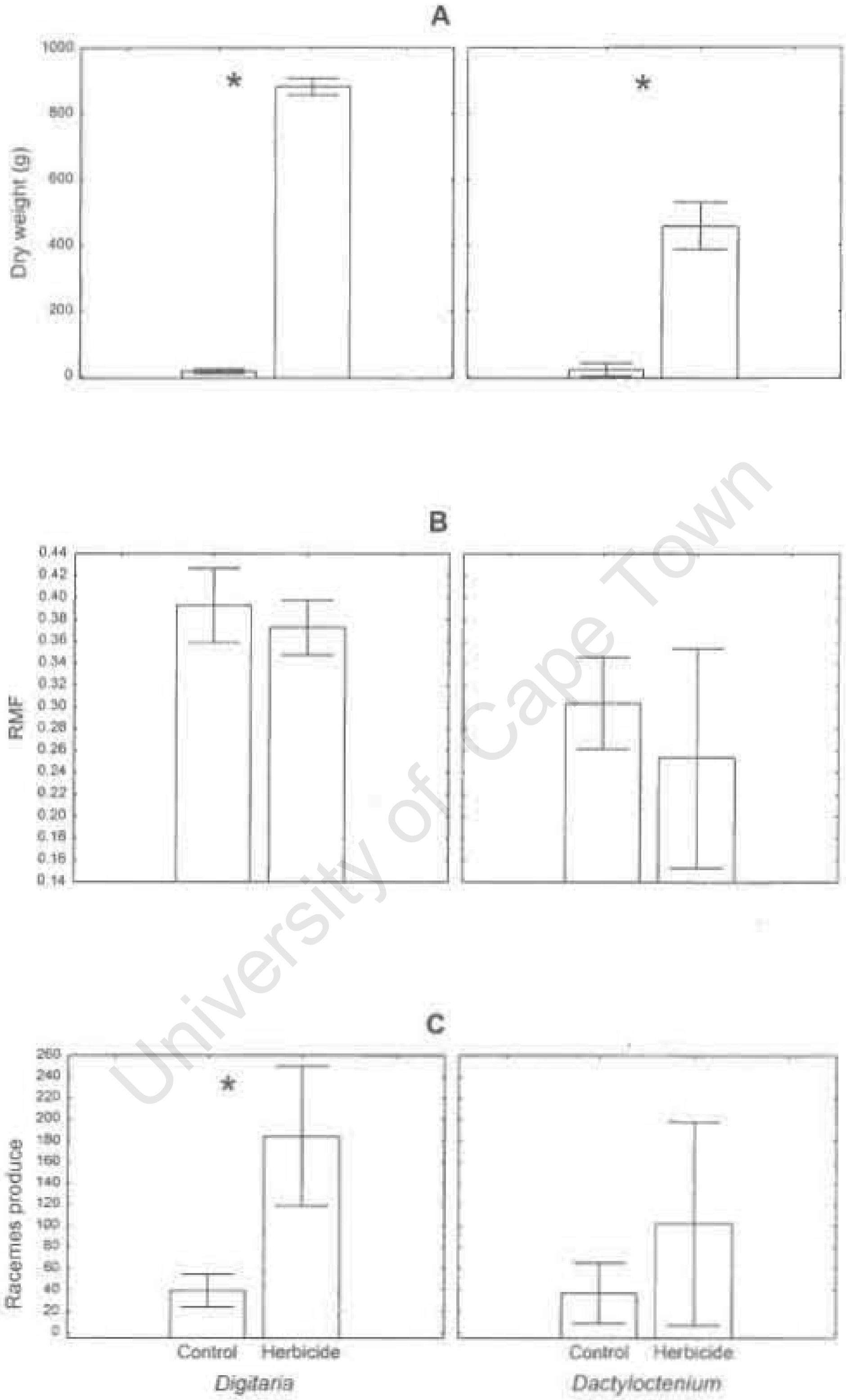


Figure 3.4. Herbicide experiment. The growth of two transplanted lawn grass species, *Digitaria* and *Dactyloctenium*, in the absence of competition at the mesic end of the rainfall gradient in Hluhluwe-Umfolozi Park. **A**. Final whole plant dry weight, **B**. root mass fraction (RMF) and **C**. total number of racemes produced by transplants in control and herbicided subplots. Bars show means and whiskers 95% confidence intervals. Asterisks show significant differences at $p < 0.05$, for treatment effects within species.

Mesic end experiment

Observations

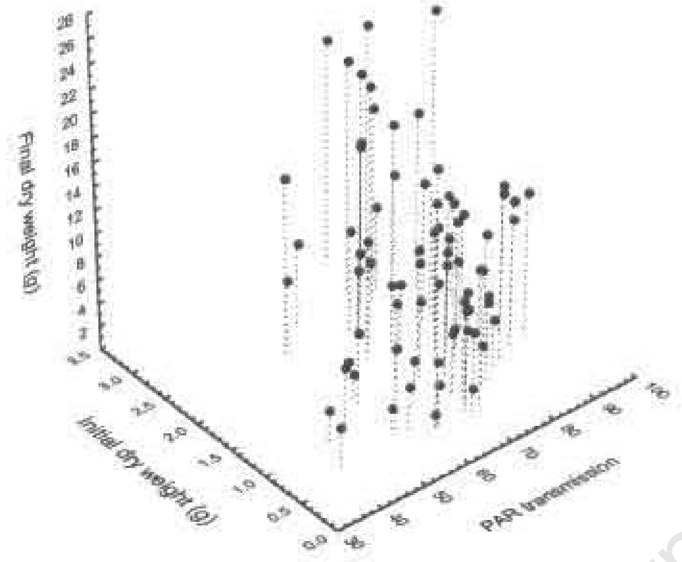
Almost all transplants showed some growth and persisted to the end of the growing season (of the few mortalities, most were due to trampling by buffalo). The morphology of the majority of *Dactyloctenium* transplants resembled that on grazing lawns, with all shoots developing into stolons. A very small proportion in the most shaded subplots adopted a 'shade growth form', with a large number of small, vertical shoots rather than a smaller number of large stolons. Most of the *Digitaria* transplants, however, adopted a 'shade growth form', and few individuals produced stolons. This was observed even in subplots in which the majority of bunch grasses were tied up.

Biomass

Although the full factorial model did provide a significant fit for *Digitaria*, none of the terms were significant. However, a reduced, additive model indicated that both initial biomass and light addition had a positive effect on final biomass for *Digitaria* (**Fig 3.5A; Table 3.3**).

For *Dactyloctenium* the effects of the treatments were more complex. The full factorial model was significant and indicated that initial biomass, N addition and light addition had a positive and multiplicative effect on final biomass (**Table 3.3**). The interaction between N addition and initial biomass appeared to be a result of fertilization only producing an increase in final biomass for transplants of intermediate initial biomass – those with lower initial biomass were little affected by fertilization, while a lack of transplants with a large initial biomass on the higher N addition levels made the relationship unclear for those with large initial biomass (**Fig 3.5B**). There also appeared to be an interaction of initial biomass and light (**Fig 3.5C**), with transplants with smaller initial biomass not responding to light addition. Finally, the interaction between light addition and N addition was a result of a lack of a N addition effect at low levels of light addition (**Fig. 3.5D**). Overall, final biomass (even on the highest levels of N and light addition) was far smaller than that in the herbicide treatment of experiment 1.

A. *Digitaria*



B. *Dactyloctenium*

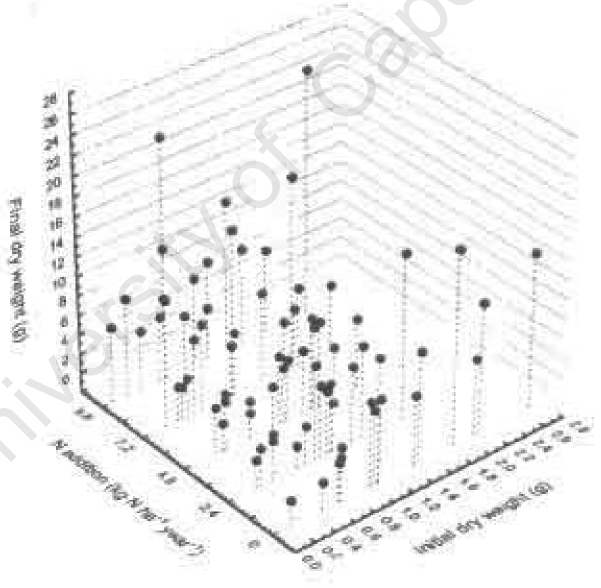
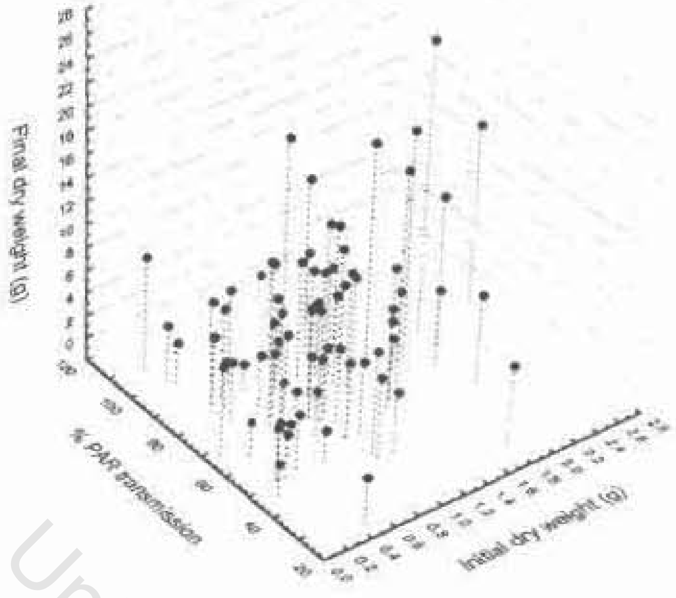


Figure 3.5. Mesic-end experiment. The effect of light and nitrogen (NH_4NO_3) fertilization on two grazing lawn species transplanted to the mesic end of the rainfall gradient in Hluhluwe-Umfolozi Park. **A**. Final dry weight of *Digitaria* versus initial dry weight and light addition (measured as percentage of PAR penetrating overhead bunch grass canopies). **B-D**) Final dry weight of *Dactyloctenium* versus **B**. initial dry weight and N addition, **C**. initial dry weight and light addition, **D**. N addition and light addition.

C. *Dactyloctenium*



D. *Dactyloctenium*

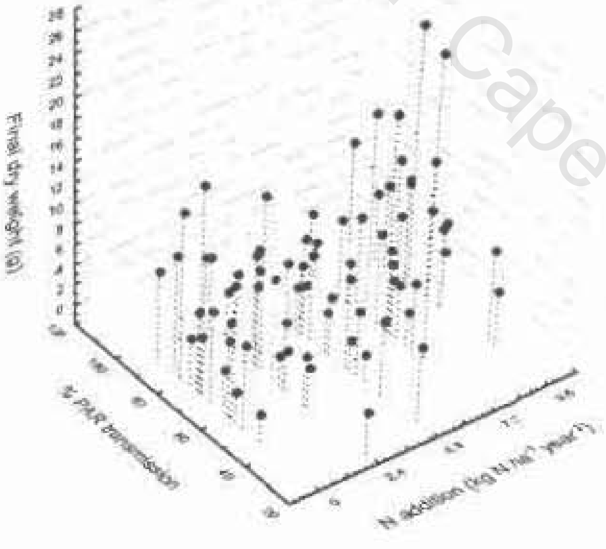


Figure 3.5. Cont'd

RMF

RMF data contained more variance, and regression models accounted for little of it. As would be expected, initial biomass had no effect on RMF. For *Digitaria*, treatment effects were similar to those for final biomass, and with light addition having a small positive effect on RMF and no effect of N addition **(Table 3.3)**. For *Dactyloctenium*, regressions models were not significant, indicating that the treatments had no effect on allocation to roots.

Raceme production

Raceme production correlated reasonably well with final biomass for both species **(Fig 3.6)**. As a result, the same terms were significant for the regressions models as for final biomass.

Table 3.3. Mesic-end experiment. Summary of the general linear models for final dry weight and root mass fraction (RMF) for *Digitaria* and *Dactyloctenium*. Full models refer to factorial models with Plot, Initial, N, L and all interactions, as terms. N = total NH_4NO_3 added; L = arcsine (PAR transmission)$^{0.5}$; Final = (Final dry weight)$^{0.25}$; Initial = (Initial dry weight)$^{0.5}$. Significance levels: * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$. MS = mean squares (effect).

	FINAL DRY WEIGHT	**RMF**
	Digitaria	
Full model	$r^2 = 0.66$; $F_{23,51} = 4.34$; $p < 0.001$	$r^2 = 0.26$; $_{F7,67} = 3.44$; $p < 0.01$
Reduced model	Final = Plot + Initial + L $r^2 = 0.49$; $F_{4,70} = 18.1$; $p < 0.001$	RMF = N + L $r^2 = 0.24$; $F_{2,72} = 11.4$; $p < 0.001$
Significant effects (MS)	Initial (0.47***) L (0.55***) Plot (0.12*)	L (0.019***)
	Dactyloctenium	
Full model	$r^2 = 0.66$; $F_{23,48} = 4.03$; $p < 0.001$	$r^2 = 0.10$; $F_{7,64} = 1.04$; $p = 0.41$
Significant effects (MS)	Plot x N (0.26**) Plot x Initial (0.23**) Plot x N x Initial (0.36***) Plot x N x L (0.23***) Plot x Initial x L (0.21*) Plot x Initial x N x L (0.35***)	

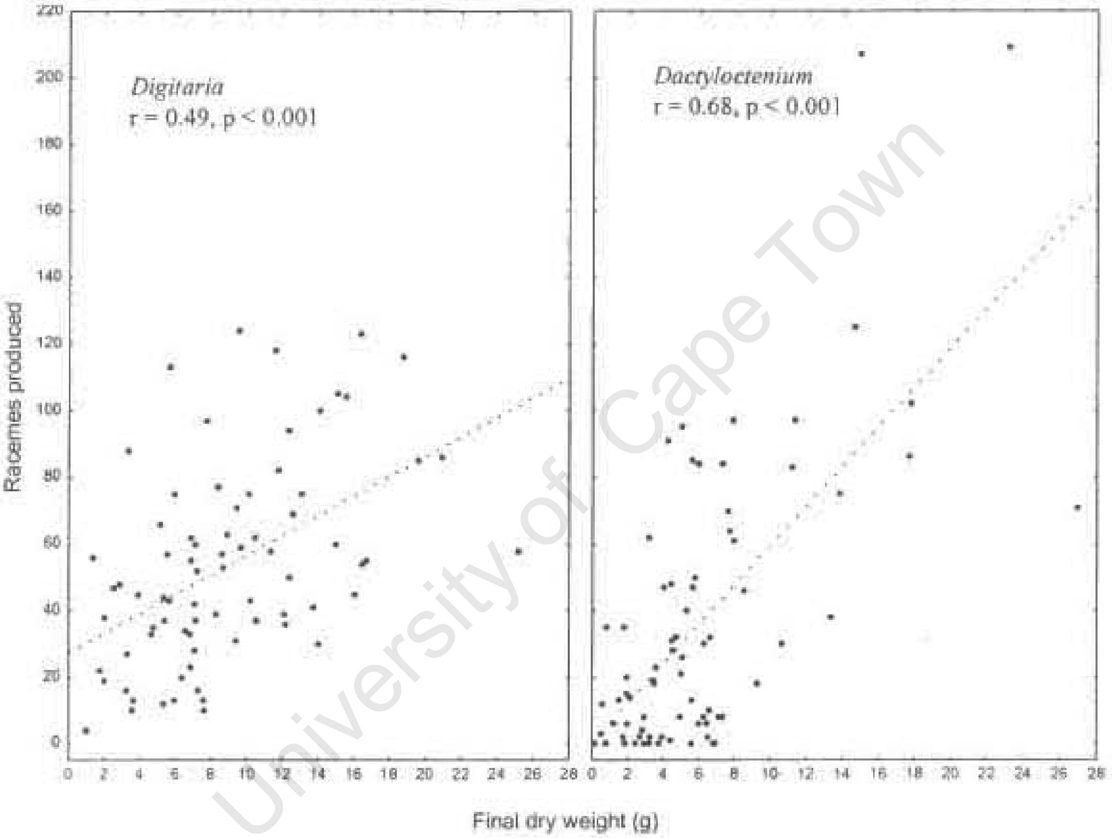


Figure 3.6. Mesic-end experiment. The number of racemes produced over the growing season versus the final biomass of two grazing lawn species transplanted to the mesic end of the rainfall gradient in Hluhluwe-Umfolozi Park. Pearson's correlation co-efficients and associated p values are shown above each plot.

Arid-end experiment

Bitten leaves

While the total number of leaves per *Urochloa* individual varied greatly through the season, the number of these bitten showed less variance, resulting in large changes in the proportion of leaves bitten (**Fig. 3.7**). While the mixed-model GLM did provide a significant fit to this data ($r^2 = 0.36$, $F_{15,228} = 8.75$, $p < 0.001$), the only term which was significant below the 10% confidence level was the date of sampling.

Crown area

Total crown area per subplot was less at the end of the growing season than at the beginning. The regression model indicated that these reductions were significantly affected by an interaction of the effects of initial crown area and irrigation (**Table 3.4**). Subplots with greater initial crown area had greater final crown area as well, but surprisingly, reductions in inter-rainfall intervals brought about by the irrigation treatment resulted in greater reductions in crown area (**Fig. 3.8**). As with the **mesic**-end experiment the interaction between the measure of initial size (initial crown area) and the significant treatment variable (irrigation) was a result of a lack of effect of the treatment on small individuals. Although N addition was not a significant term in the model, the interaction of N addition and initial crown area as well as N addition and irrigation did have relatively large effects (**Table 3.4**), and excluding N addition reduced the proportion of variance explained by the model (from 0.54 to 0.49).

Table 3.4. Arid-end experiment. Summary of the general linear model used for final crown area of *Urochloa*. The model was fully factorial, with Initial, N, W and all interactions as terms. Initial = initial crown area, N = N level, $W = e^{(\text{geometric mean inter-rainfall interval})}$. Significance levels: * $p < 0.10$, ** $p < 0.05$. MS = mean squares (effect).

Model summary	$r^2 = 0.54$; $F_{7,52} = 8.66$; $p < 0.001$
Significant effects (MS)	Initial (3072*)
	N x W (2933*)
	Initial x W (4508**)

The decrease in crown area across all subplots contrasted with the number of crowns per subplot which had increased by the end of the growing season (data not shown). This appeared to be the result of both a reduction in the size of large crowns, and an increase in the number of rooted stolon nodes (pers. obs.).

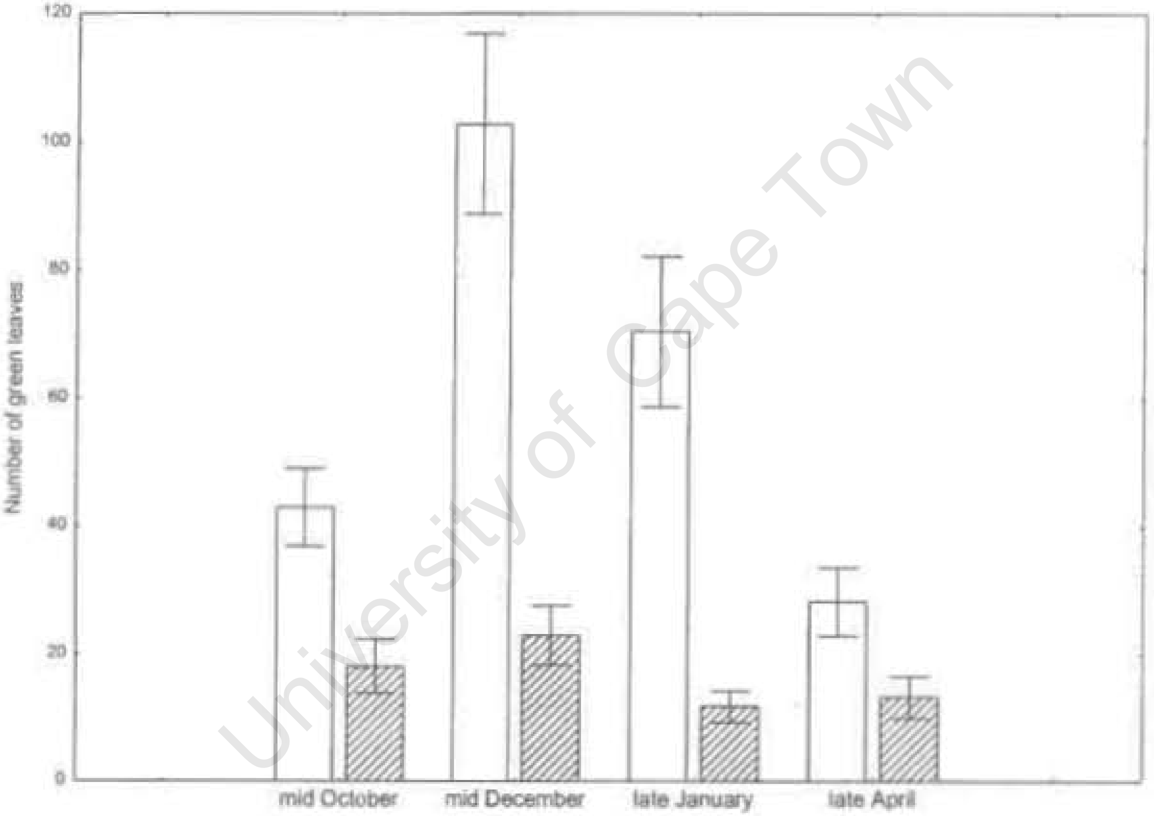


Figure 3.7. Arid-end experiment. The total number of green leaves (□) and number of these bitten (▨) on the central *Urochloa* plant in each subplot on a grazing lawn at the arid extreme of the rainfall gradient in Hluhluwe-Umfolozi Park. Data were collected on four sampling occasions through the 2000/01 growing season. Bars show means and whiskers 95% confidence intervals.

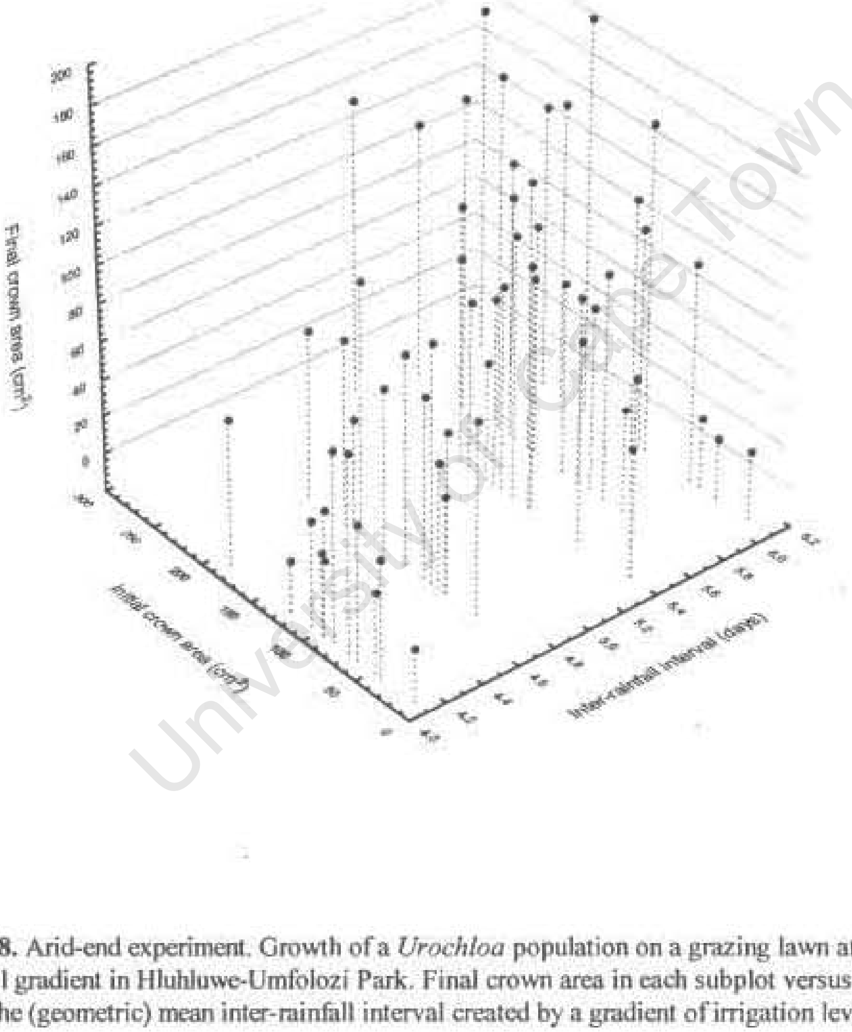


Figure 3.8. Arid-end experiment. Growth of a *Urochloa* population on a grazing lawn at the arid end of the rainfall gradient in Hluhluwe-Umfolozi Park. Final crown area in each subplot versus initial crown area and the (geometric) mean inter-rainfall interval created by a gradient of irrigation levels.

DISCUSSION

Herbicide experiment

Despite potential differences in the initial sizes of transplants, as well as the indirect measure of final sizes, the results from the **herbicide experiment** are clear. The massive increase in growth (and basal cover) of *Digitaria* and *Dactyloctenium* in the absence of bunch grasses shows that abiotic factors do not prevent the formation of grazing lawns by these two species. Competition must therefore be the primary factor limiting the distribution of these two species in the mesic parts of HUP.

Results from the analysis of the RMF data, although not significant, suggest that much of the competition that the transplanted lawn grasses experienced when grown with bunch grasses was for below-ground resources – if competition was for light only, one would expect a decrease in RMF (a response to shading shown by a wide range of species, many herbaceous - Poorter and Nagel 2000). The high variance, and resulting lack of statistical significance of this difference, may simply have been a result of the method used to calculate RMF, which was based on plants grown in a very different environment and which contained a fair amount of error itself.

While raceme production clearly followed the same pattern as final biomass, variance was again far greater. A likely reason is the grazing experienced by transplants in the herbicide subplots. While these transplants presented a fairly obvious target to herbivores, they were not grazed evenly at any one grazing event (pers. obs). Low raceme production in some subplots may therefore be an artefact of more frequent grazing in those subplots.

Mesic-end experiment

Estimation of growth

A potential problem in a transplant experiment is that acclimation and recovery from stress caused during transplanting may obscure treatment effects. This is unlikely to have occurred in the **mesic-end experiment**, as the initial measurements were done a number of weeks after transplanting, and all transplants had produced many new leaves and even culms by this stage. A more likely source of experimental error is the methods used to estimate initial and final biomass - in both cases the regressions used contained a fair amount of unexplained variance. However, there was no other practical means of estimating root biomass. The soils at the experimental site had a high clay content and invariably cracked when digging up transplants, resulting in most nodal roots breaking. The inclusion of an estimate of initial size proved vital in the regression analyses – analysis of final biomass alone indicated no significant treatment effects. Furthermore, an approximately linear relationship between initial and final sizes suggests that biomass accumulation followed a more or less linear trend across all treatment levels.

N addition

The growth responses to the rather small amounts of N added suggest that bunch grasses at the mesic extreme of the rainfall gradient in HUP do create a shortage of N for *Dactyloctenium*, but not *Digitaria*. As the growth rates of both species in this experiment were far lower than in the **herbicide experiment**, the results also suggest that bunch grass competition reduces the supply of soil resources other than N, even if grazing eliminates shading. However, as discussed below, light shortages were not properly eliminated by the light addition treatment, and it is possible that shading caused the lower growth rates even on the high light addition treatments. Also, the interactive effect of light and N found for the growth of *Dactyloctenium* might have resulted a stronger response to fertilization in an environment with more light, such as an incipient grazing lawn. Alternatively, the amount of N added may have been too small to compensate completely for N competition with the bunch grasses, and more fertilization may have produced rates of growth comparable to the herbicide experiment for *Dactyloctenium*.

The complete lack of response of *Digitaria* to N supports the results of chapter 2 (section 3) which showed that *Digitaria* has a lower 'demand' for N than *Dactyloctenium*. If *Digitaria* does not in fact experience shortage of N supply when competing with ungrazed bunch grasses, it follows that this species would certainly not experience any N shortages amongst regularly grazed bunch grasses, where N supply is likely to be higher (if any different at all). However, more careful consideration of why *Digitaria* showed no response to N addition shows that this conclusion must be treated with caution, as two alternative conclusions are plausible.

1) Light limitation

The method of light addition created a rather unnatural gradient of light supply. Firstly, the PAR transmission data showed a more or less normal distribution indicating an unbalanced light addition gradient, with a lack of replication at the low and high end. Secondly, as a result of the height to which the bunch grass canopies grew, even when tied together, the 'high' light addition treatments consisted of columns of light between tall pillars of bunch grass canopies. This appeared to create a light environment more shaded than that which would occur between grazed bunch tufts on an incipient lawn. Daily variations in light supply were probably far greater as well, with transplants experiencing either full sunlight or deep shade as the sun moved across the sky. Thirdly, the unfortunate break in the maintenance of the experiment resulted in transplants of the 'high light' treatment levels being over-topped for almost a month. Therefore, although PAR transmission was measured as close to 100% for many of these subplots, it is unlikely that the lawn grass transplant there experienced a similar light supply as that which occurs on incipient grazing lawns. The observation that most of the *Digitaria* transplants, including many in the 'high light' subplots, adopted the 'shade growth form' supports this.

It follows that shading may have prevented *Digitaria* from responding to the addition of N, even on the highest light addition treatment level. For *Dactyloctenium*, the response to N at higher PAR transmission was probably facilitated by the greater shade-tolerance of this species (suggested by its abundance under tree canopies).

2) Inability to compete for N

Another potential explanation for the lack of a response shown by *Digitaria* to N addition is that this species was unable to acquire a significant amount of the NH_4NO_3 added, despite N-limited growth. This could not have been due to competition with *Dactyloctenium* - the spacing of the transplants would have prevented any overlap of their roots. Instead, *Digitaria* transplants may have unable to take up this N before it entered one of the other potential sinks of inorganic N in the soil system (Jarvis 2000), namely:

- uptake by neighbouring bunch grasses;
- uptake by soil microbes, followed by either immobilization or denitrification (these processes are discussed further in the discussion of the **arid-end experiment**);
- leaching to soil layers below the rooting depth of the transplants.

The latter sink is unlikely as the soil at the site has a high clay content, and half of the N was added as ammonium. However, it is possible that more efficient uptake of the fertilizer by microbes or bunch grasses precluded uptake by *Digitaria*. The oversight of not measuring plant and soil N concentrations means that the importance of these two sinks in this experiment is unknown.

Light addition

The interaction of light and N supply for *Dactyloctenium* suggests that greater shading from bunch grasses on an incipient grazing lawn in the mesic areas of HUP, could contribute to the exclusion of this species. The even stronger influence of light on the growth of *Digitaria* could also indicate that shading alone reduces the growth this species on incipient lawns. Again, however, the nature of the light environments created by the light addition treatments means that these conclusions must be treated with caution. The survival of transplants in even the most shaded subplots indicates that even if intense grazing were to occur as infrequently as once a growing season, shading alone would not exclude either lawn species.

Arid-end experiment

Estimation of growth

The indirect measures of initial and final biomass were probably even less accurate in this experiment than in the **mesic-end experiment**, but again direct measures proved impossible. Error may have arisen from, firstly, the fact that it was not clear which crowns (or which parts of them) were actually alive at the beginning of the experiment. Secondly, just two measures of crown dimensions were used as a surrogate for whole plant size, although data from *Urochloa* plants grown in solution culture (chapter 2) did show a strong correlation between tuft area and whole plant dry weight ($r = 0.90$) over a wide range of size and RMF.

Changes in the number of green leaves per plant suggest that growth through the season was neither linear, nor followed a simple non-linear pattern, such as with exponential or logistic growth (**Fig 3.7**). However, the linear relationship between the initial and final measures of plant size was again assumed to indicate that biomass accumulation followed an overall linear trend regardless of treatment effects.

N addition

Assuming the above potential errors were negligible, the lack response of *Urochloa* to the fertilizer suggests that the growth of lawn grasses is not limited by N supply on grazing lawns at the semi-arid extreme of HUP. This result was surprising, considering that *Urochloa* showed such a strong response to N in solution culture (chapter 2 section 3). The optimum N concentration for this species in solution culture was between 1 and 40mM, which means that either the *Urochloa* plants in this experiment were acquiring the equivalent rate of N supply (even without fertilization) or their growth was limited by the supply of other resources. The former point is not unreasonable - data collected from a range of grass species in the semi-arid parts of HUP, including *Urochloa*, had a mean leaf blade N concentration of approximately 4% (Coetsee 2002). This is an exceptionally high value for C_4 grasses - even when fertilized with a 250 kg N ha^{-1} $year^{-1}$ *Pennisetum clandestinum*, a C_4 pasture species, was found to have approximately the same mean leaf blade N concentration (4.1% - Marais 1990) – and indicates an unusually high supply of N.

Likewise, a shortage of other resources preventing a response to N is also a plausible. Ironically, light supply may have been limiting. Although the site chosen was heavily grazed in the preceding season, direct observation, regular counts of animal dung and tracks, and the morphology of the grasses at this site indicated that grazing was infrequent during the experimental period. This was almost certainly due to the fact that the area surrounding the site did not burn in the preceding dry season, while almost 80% of the entire park did (D. Balfour pers. comm.). As regrowth on burnt areas is favoured by grazers (Vinton *et al.* 1993; Lutge 1995; van de Vijver *et al.* 1999), this probably attracted them away from the site for much of the growing season. As a result, the *Urochloa* plants developed an upright, tufted growth form and probably began self-shading early in the season. Furthermore, many co-occurring species, especially *P. maximum* and certain forb species, grew quite tall and shaded many *Urochloa* plants for much of the growing season.

The lack of grazing and associated shading lead one to question the validity of the results of this experiment. Two alternative explanations for the lack of a growth response of *Urochloa* to N addition, which allow for the possibility that this species is limited by N supply on semi-arid grazing lawns in general, are considered below.

1) Effects of more severe grazing

Would have N have become limiting with more frequent grazing? N demands may increase with defoliation (chapter 2 section 1) and light supply (Lambers *et al.* 1998). Therefore, it is possible that the growth of *Urochloa* is limited by shading in the absence of grazing, but limited by a shortage of N when frequent grazing removes eliminates shading.

2) Ability to compete for N

As was argued for *Digitaria*, it is possible that *Urochloa* plants did experience N shortages, but were unable to acquire any of the added NH_4NO_3. Of the three alternative sinks for the added N mentioned above, only the microbial one is likely to have been strong enough to divert N from uptake by *Urochloa*. Leaching is again unlikely, due to the reasonable high clay content of the soils. Uptake by co-occurring plants is also unlikely to have been important, as *Urochloa* was by far the most abundant species at the site. In addition, the next most abundant species, *P. maximum*, has shown a lack of response to NH_4NO_3 fertilizer, even at very high levels, in a long term fertilization experiment (O'Connor 1999). This leaves the possibility that all added N was immobilized and / or denitrified by soil microbial activity.

Immobilization

Studies which have analysed the fate of added N in the plant-soil system by means of labelling fertilizer with the ^{15}N isotope show that immobilization of added N is common. However, all these studies indicate at least some uptake by target plants as well. In a Colorado semi-arid grassland, approximately 25% of the 1g N m^{-2} added (as $^{15}NH_4{}^{15}NO_3$) was taken up by grasses after 1 month, and approximately 33% by the end of the growing season (Epstein *et al.* 2001). Working in the same ecosystem, (Schimel 1986, cited by Epstein *et al.* 2001) found between 11 and 14% uptake after first growing season. In temperate, intensively grazed C_3 grassland, Ledgard *et al.* (1998) report that 27% of N (added as NH_4SO_4) had been taken up by grasses after only 3 days, with only approximately 10% immobilized as microbial biomass. Finally, immobilization of N from ^{15}N labelled urea applied to a wheat field accounted for only 13-16% of the N applied (Recous and Machet 1999). These results suggest that a certain proportion of the N added would not have been available to *Urochloa*, but whether this means that this species still experienced N shortages is unknown.

Denitrification

N losses through denitrification from heavily grazed grasslands can increase dramatically after fertilization, but never account for a significantly large portion of the N added (Jarvis 2000). Furthermore, the results of Frank and Groffman (1998a) suggest that denitrification is not of much significance in semi-arid environments.

While the above points may not be valid for this experiment, they show that the conclusion that *Urochloa* is not limited by N supply on the semi-arid grazing lawns of HUP must be treated with caution. The experiment would need to be repeated, under conditions of a greater grazing intensity, to accept this conclusion with confidence. Tracking the fate of the N added, as in the studies cited above, would be useful for interpreting results (more so than measuring leaf N concentrations, which would not indicate whether target plants were outcompeted for additional N, or simply lacked the ability to acquire it due to a shortage of other resources).

Irrigation

The irrigation treatment of experiment 3 was far too subtle. The maximum level, intended to reduce the ambient mean inter-rainfall interval by half, only achieved a reduction of about one fifth (**Table 4.2**). In addition, due to the unanticipated interruption of the irrigation schedule, all treatments levels ended up with a far greater range in inter-rainfall interval than intended (up to 18 days). The fact that irrigation still caused a response, but one completely opposite to that expected, is very difficult to explain.

Two factors likely to be responsible for this result are the above-average rainfall and the unusually low grazing at the experimental site during the 2000/01 growing season. The mean growing season rainfall from the 10-year data set was 568mm, while 622mm was recorded during 2000/01. In addition, the average inter-rainfall interval was less that half that from the 10-year data set. Together with the lack of grazing, this resulted in marked vertical growth of both co-occurring grasses and forbs, which may have suppressed the growth of *Urochloa* (either through shading or competition for soil resources other than water and N).

The results of the irrigation treatment therefore provide insight into how lawn grass growth in a semi-arid environment is affected by above-average water, and below-average grazing intensity. The fact that this led to reduced cover of a grazing lawn species supports the grazing-lawn model presented in chapter 1. However, it does not indicate whether water would be limiting in a growing season with average or below-average rainfall. As with the fertilizer treatment, the irrigation would need to be repeated, in a drier year and with a more rigorous irrigation regime.

Conclusion

Of the three experiments performed, only the **herbicide experiment** provided a conclusive result, namely that competition rather that abiotic factors exclude lawn grasses from the most mesic parts of HUP. The N addition treatments of the **mesic-** and **arid-end experiments** suggest that N shortages may limit the distribution of lawn grasses at the mesic end of the rainfall gradient only, and then only for certain species. This conclusion is tentative, as it cannot be said whether the two species which did not respond to the fertilization (*Digitaria* and *Urochloa*) were prevented from doing so by a shortage of other resources or more efficient uptake by other species. The light addition treatment of the **mesic-end experiment**, while not well designed or implemented, did reveal that both species are influenced negatively by shading but not to the point that this would prevent their survival between grazing events. Finally, the irrigation treatment of the **arid-end experiment** provided no insight into whether *Urochloa*, a common lawn species at the arid end of the rainfall gradient in HUP, is excluded from the heavily grazed areas by a shortage of water.

CHAPTER 4

Population growth patterns of two mesic lawn species

INTRODUCTION

This chapter investigates whether the evidence for bunch grass competition reported in the previous chapter are relevant to the growth of lawn grasses in the heavily-grazed, mesic environments of HUP. If these results are applicable, one would expect significant growth increases in response to the elimination of below-ground (BG) competition for *Dactyloctenium*, and above-ground (AG) competition for *Digitaria*, (when growing amongst heavily-grazed bunch grasses). Alternatively, as the previous experiment considered BG competition in the form of N supply only, either species might respond to the elimination of all BG competition as a result of an increased supply of another resource. To test for the effects of both BG and AG competition imposed by grazed bunch grasses, a root trenching experiment was designed. In addition, a non-manipulative experiment was performed to determine whether the intensity of bunch grass competition differs between drier areas of HUP (where grazing lawns are common) and the most mesic parts (where they are all but absent). This simply involved measuring the growth of established lawn grasses at different sites at a range of altitudes.

Experimental designs

In chapter 2, it was hypothesized that BG competition, for nitrogen in particular, limits the distribution of lawn grasses in the more mesic parts of HUP. Chapter 3 introduced the idea that even with heavy grazing, lawn grasses could experience competition for light, as the re-growth of bunch grasses between grazing events could be great enough to begin shading them. For the trenching experiment a design was required which could eliminate one of each type of competition (AG or BG) without interfering with the intensity of the other. However, as was learned from the mesic-end experiment performed in the previous chapter this is extremely difficult to achieve, if not impossible. As a compromise, an additive (rather than factorial) designed was used.

The design aimed to eliminate either BG competition imposed by grazed bunch grasses through the use of root trenches, and both AG and BG competition through the use of root trenches and the removal of all AG bunch grasses parts. It was expected that the importance of BG competition to lawn grasses would be revealed by differences in growth between the control and 'trenching only' treatment, and the importance of AG competition by differences between the 'trenching only' and the 'trenching and clearing' treatments.

Response variables

All previous experiments of this thesis have measured growth as short-term (i.e. less than a year) biomass production and flower production. Short-term biomass production may be the best measure of **within-season** growth, but for environments where growth is annually interrupted by a dry season it introduces the question of how much biomass produced in one growing season persists to the following growing season. As the topic of this thesis concerns a pattern of lawn grass distribution which has persisted for many years (if not centuries), it is the carry-over of growth from year to year (referred to as **multi-season** growth hereafter) that is of prime importance. Measuring biomass accumulation accurately also requires estimates of initial biomass (i.e. biomass at the start of the growing season) which proved to be very difficult to measure in the previous field experiment.

Therefore for the two experiments of this study, alternative measures of growth were employed. **Fig. 4.1** shows all the pathways by which an established lawn grass genet (i.e. a single genet individual) could grow. For within-season growth, many pathways are possible for plants of a clonal growth form, such as the two lawn grass species used in this study. All of these could result in storage of assimilates by the crown of an established genet, which is known to contribute to multi-season growth, (although for species of different growth form, Grime 2001). However, most within-season growth will be lost, as shoots, stolons and flowers do not persist through the dry season for the species in question (pers. obs.).

Only selected pathways in **Fig. 4.1** result in reproduction (sexual or vegetative) which would contribute the most to multi-season growth, as it enables the exploitation of resources at multiple points in space. In addition, for lawn grasses the resulting increase in horizontal cover (spread) may increase the attraction of grazers to an incipient lawn, thus reducing competition and increasing growth further. There are two reproductive pathways: the production of rooted stolon nodes which persist to the following growing season, and the production of seeds which germinate successfully the following growing season. Genetic analysis of clonal mats of the grazing lawn species *Andropogon greenwayi* in the Serengeti suggest that lawn grasses reproduce sexually fairly regularly (Belsky 1986a). However, it is the production of rooted nodes which is of particular interest - this has been shown to be important for multi-season growth (under conditions of heavy grazing) for another southern African stoloniferous species, *Digitaria eriantha* (O'Connor 1991b), while seed recruitment has been shown to be of very limited importance for a number of grazing-tolerant species (Belsky 1986b; O'Connor and Pickett 1992). The production of rooted nodes might also contribute directly to within-season growth through the exploitation of resources distant to a genet's original crown, and possibly by increasing grazing pressure. However, it is only when rooted nodes remain alive until the following growing season that they would contribute directly to multi-season growth.

Ideally, one would measure each pathway depicted in **Fig. 4.1**, including direct measures of reproduction the following season. However, time constraints meant the measurements could only be taken within a single growing season, while practical constraints precluded measurements of storage. In the end direct measurements of only some of the within-season growth components were measured, and surrogates
used for only some of the multi-season growth components (see **Fig. 1**)

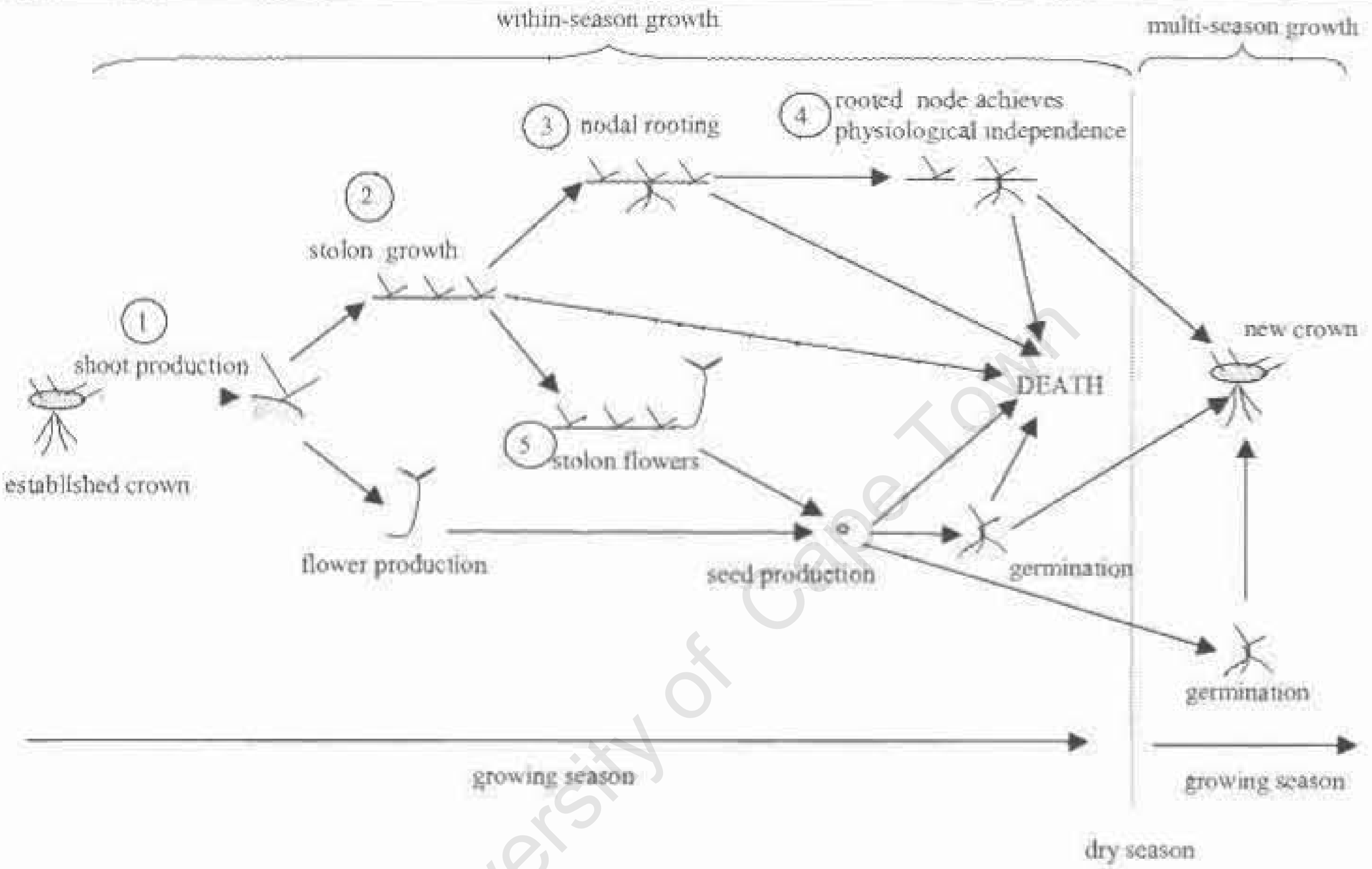


Figure 4.1. Schematic representation of all possible growth components for a lawn grass genet. All pathways within a growing season could increase growth of the parent crown in the following growing season, through the storage of assimilates. For only a few pathways does growth continue into the following growing season, resulting in a inter-annual reproduction of the parent crown. Numbered pathways are those for which measurement was attempted.

METHODS

Species

For both experiments the two mesic lawn species *Digitaria longiflora* and *Dactyloctenium australe*, used and described in chapters 2 and 3, were used (again, they are referred to by their generic names).

Sites

1. Trenching experiment

Separate sites were used for each species. The site for *Dactyloctenium*, Mbombe, was located on the crest of one of the highest hills in HUP (**Fig. 4.2A**, see **Fig. 1.1** for location). It consisted of three patches of naturally occurring lawns (approximately 10 x 10m) dominated by *Dactyloctenium*. The soil and the composition of the surrounding bunch grass community are the same as that described for experiment 1 and 2 in chapter 3. Afromontane forest lined the top of the hill above the site, and at some points was only a few meters from two of the *Dactyloctenium* lawns. Although no trees over-topped these patches, they did experience some shading in the late afternoon. Other species recorded on the lawns were *Chloris gayana*, *Themeda triandra*, *Bothriocloa bladhii*, *Hyparrhenia filipendula*, *Sporobulus africanus* and *Sporobolus pyramidalis*.

For the *Digitaria* experiment an artificial lawn was created as no *Digitaria*-dominated lawns of adequate size could be found at the mesic extreme of HUP. The site chosen (Mbombe High 1) ran along the top of a ridge, about 100m down slope of the Mbombe site (**Fig. 4.2**). It consisted of a large patch (approximately 20m x 20m) of shortly-grazed bunch grasses (approximately 5cm tall), which appeared to have been heavily grazed for at least the previous season. A number of depressions of bare soil indicated frequent visits by large herbivores (the depressions being used for rolling, scent marking or territorial displays). Soil at the site was clayey, as at Mbombe, but shallow and rocky. The dominant species was *Hyparrhenia filipendula*; others recorded were *Themeda triandra*, *Aristida bipartita*, *Chloris gayana*, *Tristachya leucothrix*,

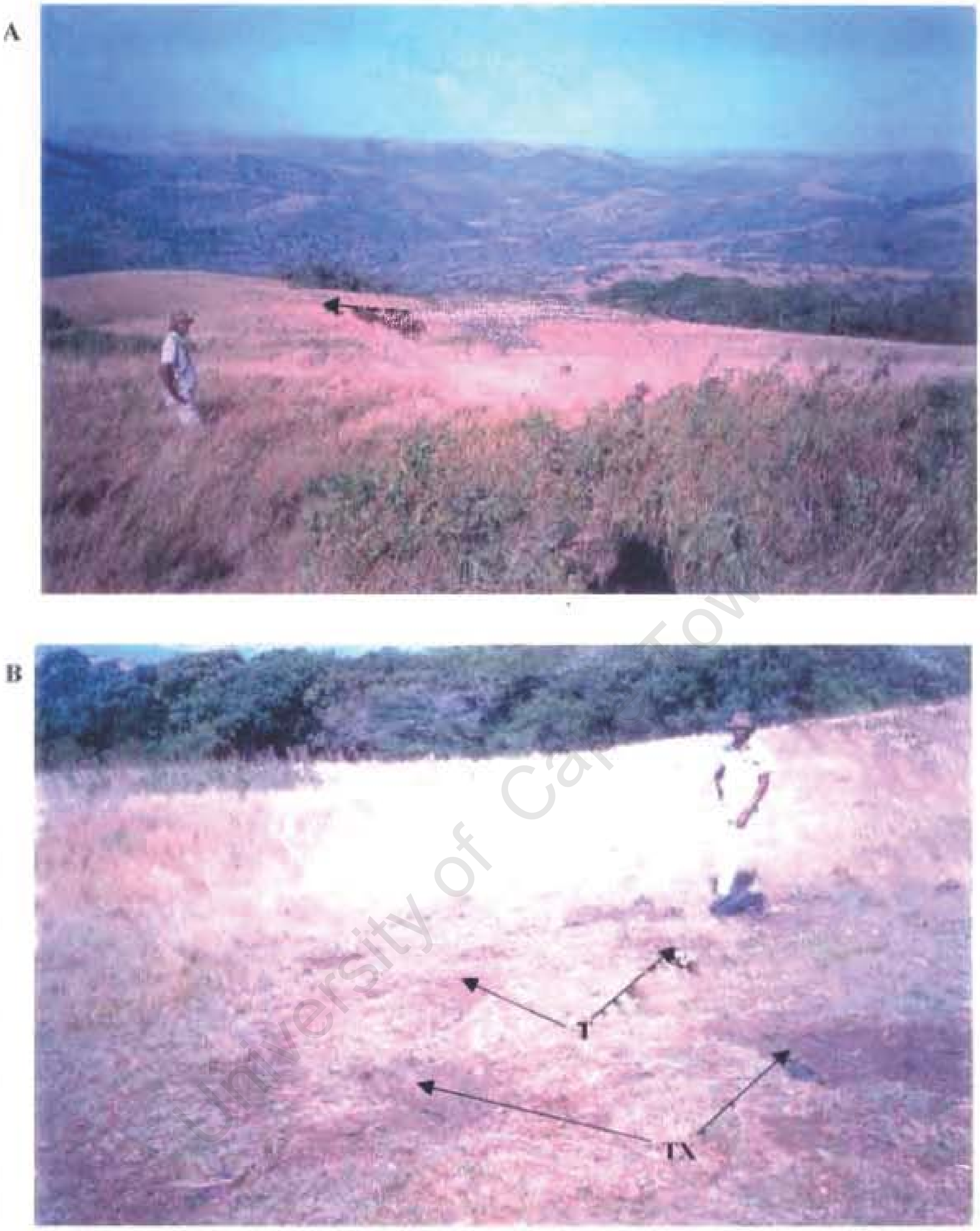


Figure 4.2. Sites used for measuring the growth of lawn grasses at the upper end of the altitudinal gradient for the landscape experiment conducted in Hluhluwe-Umfolozi Park, South Africa. **A.** A photograph taken just below the Mbombe site, looking down the Mbombe ridge. The arrow shows the location of the Mombe High 1 site. **B.** The Mbombe High 1 site at the end of the growing season (April 2002). Arrows show transplants of *Digitaria longiflora* within pairs of plastic-lined root trenches used for the trench (T) and 'trench and clear' (TX) treatments.

Paspalum scrobiculatum, *Eulalia villosa* and *Panicum coloratum*. The surrounding ungrazed bunch grass community was the same as that at Mbombe.

Individual *Digitaria* tufts were transplanted from two small (2 x 2m) *Digitaria*-dominated lawn patches located nearby the Mbombe site. 30 individuals were transplanted in late September 2001. A few which did not survive were replaced in early October. See **Table 4.1** for the altitude and an estimate of rainfall for these sites.

2. Landscape patterns

For *Dactyloctenium*, a series of sites were located downslope from the Mbombe site. These sites were all patches of *Dactyloctenium*-dominated lawns, at least 2m in diameter and were not directly shaded by trees. One (Mbombe Mid 1) was directly adjacent to a warthog burrow, suggesting a long history of intense grazing. Another (Mbombe Mid 3) consisted of three lawn patches around a white rhino midden and wallow. Two further sites (Nombali 1 and Dunngamizi) were chosen many kilometres south of the Mbombe hill, in a more arid part of HUP where *Dactyloctenium* lawns are common and more extensive. This resulted in a series of sites down an altitudinal gradient, with the control plants of the trenching experiment at Mbombe representing the highest altitude site.

No *Digitaria*-dominated lawns were found further down the Mbombe hill, and an altitudinal gradient of sites could not be established. The sites included therefore represent the high and low end of the altitudinal gradient only. Again, the control plants of the trenching experiment were used as the highest altitude site (Mbombe High 1). These were supplemented by two small patches of naturally occurring, *Digitaria*-dominated lawns. One (Mbombe) was approximately 100m upslope from Mbombe High 1 and consisted of the two patches from which transplants were taken for the trenching experiment. The other (Mbombe High 2) was approximately 200m down slope from Mbombe High 1, and consisted of a small patch of *Digitaria* adjacent to both a rocky outcrop and another bare depression. In addition, a small group of *Digitaria* individuals was found amongst a sward of unburnt, ungrazed bunch grass, less than 50m from the Mbombe High 1 site. This was included as an additional high altitude site. For the low-altitude end of the gradient, three sites were chosen in a more arid part of HUP where *Digitaria* lawns are most common. One of these (Nombali 1) was the same site used for *Dactyloctenium* above – its consisted of a large (approximately 30 x 30m) lawn with both

Digitaria- and *Dactyloctenium*-dominated patches. Another (Nombali 2) was located adjacent to the control area of the Nombali grazing exclosure of the Zululand Grasslands Project. The third (Crossroads) was located on a large (20 x 20m) lawn. All sites had dolerite bedrock (Research 1979) and clay-rich soil (types unknown). Altitudes are given in **Table 4.1**.

Experimental design

1. Trenching experiment

At each site a control (**C**) and two trenching treatments were created, with 10 replicates each. The trenching treatments were:

1. Trenching only (**T**): a narrow trench at least 15cm deep and 30cm long was dug on both sides of an established lawn grass crown, in the direction of a young, growing stolon (**Fig. 4.3**). Trenches were dug at an angle of no more than about 30° to the direction of slope, so as not to interrupt any lateral movement of soil water downslope. Thick polyethylene sheeting was inserted in each trench and soil replaced on the side of the plastic furthest away from the stolon. All forbs and bunch grasses were removed from the strip between a pair of trenches by scraping with a pick (this removed crowns but left the roots.

Table 4.1. Summary of the environmental variables measured at sites in HUP where stolons of *Digitaria* and *Dactyloctenium* were marked and routinely measured. Altitude was determined using a handheld GPS or estimated from a 1:50 000 topographic map. For each species, the proportion of stolons bitten in each interval, was not significantly different between sites (Kruskal Wallis test). Mean distance to bunch grasses is for measurements to eight nearest bunch grasses for each stolon at a site. For each species, different superscripts show significant differences between sites, according to a HSD post-hoc test of a nested ANOVA (see **Table 4.5**). Rainfall was recorded at nearby weather stations, from mid-September 2001 to mid-April 2002.

Species		**Site**	No. stolons marked	Altitude (m.a.s.l.)	Growing season rainfall	Distance to weather station (km)	Mean distance (cm) to bunch grasses (SD)	Median proportion of stolons bitten per interval (range)
Digitaria longiflora	1	Mbombe	10	505	622	2	35.1^{b} (23.2)	10 (0 – 50)
	2	Mbombe High 1 (ungrazed)^{1}	10	± 480	622	2.5	12.8^{a} (6.16)	0 (0 – 30)
	3	Mbombe High 1*	10	± 480			9.66^{a} (3.78)	7.4 (0 – 56)
	4	Mbombe High 2	10	± 400			31.6^{b} (13.3)	0 (0 – 67)
	5	Nombali 1	10	240	392	0.5	74.4^{d} (26.4)	0 (0 – 33)
	6	Nombali 2	15	205	392	0	69.4^{d} (23.5)	6.7 (0 – 33)
	7	Crossroads	10	130	392	1.7	53.7^{c} (24.0)	10 (0 – 22)
Dactyloctenium australe	1	Mbombe*	10	505	622	2	22.1^{a} (15.7)	6.9 (0 – 33)
	2	Mbombe High	10	± 480	622	2.5	24.7^{ab} (12.1)	0 (0 – 20)
	3	Mbombe Mid 1	10	370			25.4^{ab} (10.7)	10 (0 – 14)
	4	Mbombe Mid 2	10	360			30.7^{b} (12.0)	0 (0 – 20)
	5	Mbombe Mid 3	15	320			46.0^{c} (42.2)	13.3 (0 – 30)
	6	Mbombe Mid 4	15	270			32.8^{b} (12.4)	6.7 (0 – 33)
	7	Nombali 1	10	240	392	0.5	60.7^{d} (32.1)	10 (0 – 60)
	8	Dunngamizi	15	195	392	1.5	59.7^{d} (22.3)	0 (0 – 14)

* sites used for trenching experiment

[1] only site where bunch grasses were ungrazed and overshadowed lawn grasses

of the removed plants behind). The aim of this treatment was to exclude BG competition between the marked stolon and surrounding bunch grasses.

2. Trenching and clearing (**TX**): trenches were dug as above. In addition, all forbs and bunch grass in a 30cm border strip on the outside of the trenches was cleared as well (**Fig. 4.3**). The aim here was to eliminate any possible shading by surrounding bunch grasses and thus eliminate both BG and AG competition.

Trench pairs were extended as necessary, when stolons grew exceptionally long.

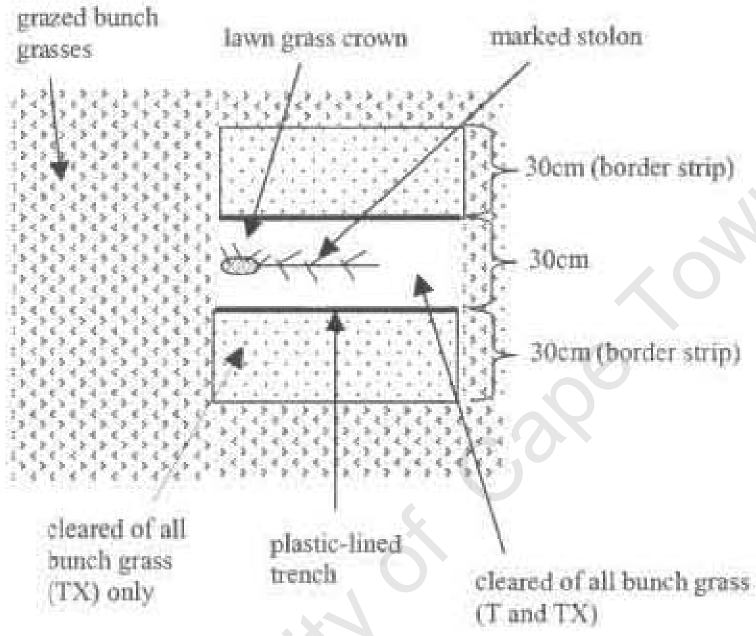


Figure 4.3. Trenching experiment. Layout of the trenches and areas cleared around each marked stolon for the T (trench only) and TX (trench with border strips cleared). Trenches were approximately 15cm deep and lined with polyethlyene sheeting.

In order to determine the effectiveness of the root trenching, root density was measured at the end of the growing season. This was done by taking root cores (depth 10cm, diameter 5cm) in early April 2002. Ten cores were taken from inside trenches (either T or TX), where no stolons had rooted, and ten between bunch grass crowns in the surrounding control area. Roots were washed and separated from soil with the use of a sieve (2mm mesh), dried for 2 days at 70°C and weighed to 2 significant figures. While it is live root which was of interest, it proved impossible to distinguish live roots from dead roots without microscopic examination (this was due to the organic content of the soil which appeared to dye all roots the same brown colour, regardless of their vitality). Root density was therefore calculated as the dry weight of all root matter per cm^3 of soil.

In order to estimate the degree to which the TX treatment reduced shading, the height of bunch grasses was measured alongside both T and TX trenches, as well as at random points around control individuals. This was done on the 19/12/2001, in the middle of the growing season.

Grazing intensity was very low during the first half of the growing season, apparently as a result of a fire in early September 2001 which burnt most of the Mbombe hill, but not the area immediately surrounding Mbombe and Mbombe High 1. This led to a substantial bunch grass canopy developing and a level of shading that was not anticipated, particularly for the *Dactyloctenium* lawns at Mbombe. Both sites were therefore mowed to a height of approximately 5cm after the grass heights measurements on the 19/12/2001. Mowing thereafter proved unnecessary, as the majority of the bunch grasses remained at this height (this appeared to be the result of both lower rainfall in the second half of the growing season, and an influx of grazers in response to the mowing).

Landscape patterns

The landscape pattern experiment involved no manipulation of growing conditions. Rather differences in altitude were assumed to create differences in the abiotic environment (which in turn was assumed to create differences in the intensity of competition imposed by bunch grasses). Measurements were made on established lawn grasses at each of the sites selected (see **Table 4.1** for replication). The following environmental variables were measured:

1. **Rainfall**, at the high and low altitude sites only. This was estimated from the amount of rainfall recorded for the 2000-2001 growing season at nearby weather stations. For low altitude sites, data was obtained from an automated weather station at the Nombali 2 grazing exclosure mentioned above. For the high altitude sites, data was obtained from the Hluhluwe Research centre, which lies at approximately the same altitude as the Mbombe site.
2. **Distances** from established lawn grasses to the eight nearest bunch grass tufts, as an estimate of the intensity of both AG and BG competition. Only bunch grasses with a crown larger than 2 x 2cm (major and minor axes) were included.
3. **Root density**, at high and low altitude sites only. The root mass data collected from control areas in the trenching experiments were used for *Dactyloctenium* at Mbombe and *Digitaria* at Mbombe High 1. 10 root cores were also taken in ungrazed bunch grass swards surrounding each of these sites. For the low-altitude sites, root mass density data

was obtained from the Zululand Grassland Project control plot at Nombali 1. This data was collected in the immediate vicinity of the Nombali 1 site on the 25th March 2002 in the same manner as described above.

4. **Grazing intensity**, as the proportion of marked stolons which were bitten.

Growth measurements

For both the trenching and landscape pattern experiments, the same growth variables were measured, and the same response variables calculated. Stolons were marked with a small piece of plastic coated wire, which was twisted loosely around the first internode. Young stolons, as short as could be marked in this way, were chosen. The following measurements were made, at regular intervals through the growing season:

1. The number of other shoots on the parent crown of the stolon (this included all shoots, not just those with culms or stolons).
2. Stolon length, measured from the junction with the crown to the distal node (to the nearest 5mm).
3. The number of nodes which were rooted.
4. If the stolon had been bitten, or had developed into an inflorescence, this was recorded.

Measurements began soon after the first rains of the growing season when all grasses were green and growing. The date of first measurement varied from 20th September 2001 at the higher altitude sites, to the 10th October the lower altitude sites. Intervals between measurements ranged from 8 to 21 days. Final measurements were taken between the 5th and 9th April 2002 at all sites.

If stolons had disappeared, or had flowered, a new stolon was chosen, as close as possible to the original, and marked and measured as above. If stolons had been bitten, this was noted but measurements continued. If a stolon had grown exceptionally long or had a severed internode, a final measurement was made and a new stolon was marked and measured as above.

Response variables

Reproduction variables

Flowering was estimated as the proportion of all stolons which produced an inflorescence. Vegetative reproduction, in terms of the rate at which stolons produced physiologically independent (rooted) nodes could not be estimated directly. Rather, the proportion of marked stolons which had rooted nodes at the time of last measure was used as the variable. This assumes that firstly, one (and only one) of the rooted nodes on these stolons would persist through the dry season until the following growing season. Secondly, the last interval of the study was not performed right at the end of the growing season, and rainfall did occur shortly thereafter. Therefore, it is assumed that the frequency of stolons with rooted nodes did not change after the last measurement interval. Stolons which grew particularly fast and were replaced during the growing season were also included.

Shoot production and stolon elongation

For both the rate of shoot production and stolon elongation, growth curve analysis (*sensu* Potvin *et al.* 1990) was not be attempted as graphical analysis showed that the pattern of change of both shoot number and stolon length with time was complex and involved many erratic changes (including negative growth). Instead, a repeated-measures ANOVA approach was adopted (von Ende 1993). This involved calculating the rate of shoot production, and stolon elongation, which occurred within each measurement interval:

rate = (shoot no. at t_2 – shoot no. at t_1) / interval length (days)

(and likewise for stolon length)

This assumes that changes in shoot number, or stolon length, that occurred within an interval were linear with respect to time (whether positive or negative) - stolon elongation has been shown to be very close to linear for two other grass species (Cruz and Boval 2000). Stolons which had died back completely were not included (i.e. if the entire stolon had died there was no value for elongation for the interval in which it died).

For the trenching experiment, differences in defoliation between treatments were observed. It appeared that with all surrounding grasses cleared, the marked stolons presented a clear target to grazers (which included warthogs, buffalo, white rhino, and blue wildebeest). In order to remove the potentially confounding effect of grazing, all interval-stolon combinations for which a bite was recorded were excluded from the calculations of stolon elongation for this experiment.

Rooted node production

A growth curve type of analysis was again precluded due to the complex patterns of change in rooted node numbers. In addition, the replacement of stolons through the season may have confounded treatment effects, as stolons which grew faster were more likely to be replaced, but may also have produced more rooted nodes (per unit length). Therefore, a repeated measures approach was adopted as above. The number of rooted nodes per unit stolon length was calculated, for each marked stolon, for each interval. This enabled analysis of how treatments affected the number of rooted nodes produced per stolon within each interval, although the approach does assume that the production of rooted nodes is independent of stolon length.

For the trenching experiment, bitten stolons could not excluded, as stolons with rooted nodes had a high probability of being grazed, and their exclusion left too few replicates for statistical analysis.

Stolon survival

Stolon survival was calculated as the number of days between the first date of measurement and the date at which a stolon was recorded as dead (or had disappeared). As only short stolons were selected when doing initial measurements, this is likely to have approximated stolon life-spans reasonably well.

Statistical analysis

All statistical analyses were performed using Statistica 6.0 (StatSoft 2001)

Environmental variables

Trenching experiment

Root mass density data were slightly skewed, but did not show significantly different variance between trench and control groups, and were analysed with a student's T test. Grass height data were heavily skewed to the right for all treatments, with some of the TX groups consisting only of 0's. Data were therefore rescaled to eliminate zeros, and analysed with a Generalized Linear Model (GLZ) one-way ANOVA, using a separate model for each site. A gamma distribution function with an identity link was used, to account for the skewed distributions. Goodness of fit was assessed on the basis of the distribution of residuals, and deviation of the Pearson Chi-squared statistic, deviance statistic and log-likelihood values from 1 (StatSoft 2001).

Landscape pattern

Grazing intensity

Although all sites had bitten stolons, at one interval or another, the frequency distribution of the proportion of stolons bitten was skewed left, with modes always equal to zero. Normality could not be achieved through standard transformations, while a GLZ Model ANOVA with a gamma distribution did not provide a satisfactory fit to the data (a model not suitable for repeated measures analysis in any case). While the non-parametric Kruskal-Wallis test does not account for repeated-measures either, it was employed to test for very large differences between sites.

Distance to bunch

Data were root transformed to improve normality and eliminate increases of variance with means. This also decreased heterogeneity of variance between groups, although this remained significantly high (as tested with the Bartlett *Chi*-squared test). For each species, differences between sites were tested using a General Linear Model (GLM) nested ANOVA, with data for each marked stolon nested within site. Post-hoc differences were tested using the unequal N HSD test.

Root mass density

Although not directly relevant to the experiment, root mass density data for ungrazed swards at Mbombe and Nombali 1 were included in the analysis. Data were ln transformed to meet assumptions of normality and tested with a GLM one-way ANOVA.

Response variables

Each response variable was analysed in the same manner for each experiment-species combination.

Reproduction variables

Differences in the proportion of marked stolons which flowered, and the proportion which had rooted nodes at the time of last measurement, were tested with Chi-squared analysis, using the Log-linear Module of Statistica v6.0.

Shoot production and stolon elongation

The fact that marked stolons often disappeared and were regularly replaced resulted in an unbalanced design, with the number of replicates varying from treatment to treatment and from one interval to the next. This precluded the use of a repeated-measures ANOVA. Instead a Mixed Linear Model (MLM) was used, which effectively allows for the use of ANOVA models for unbalanced data containing random effects. To account for the repeated measures design, a GLM model with the following terms was used (following von Ende 1993):

- shoot production / stolon elongation for each marked stolon (random effect, nested within treatment)
- treatment (fixed effect)
- interval (fixed effect)
- 'treatment x interval' (fixed effect)

Shoot production

Data were approximately normally distributed, but showed severe kurtosis. Standard transformations do not eliminate this, and raw data were used for both experiments, for both the *Digitaria* and *Dactyloctenium* analyses. In all cases, the assumptions of homogeneity of variance between groups was violated, but there was little increase in variance with means.

Stolon elongation

For the trenching experiment, data were skewed right for *Digitaria*, particularly for the control treatment (this seemed to be a result of excluding bitten stolons – *cf.* landscape pattern analysis below). Nevertheless the assumptions of normality were upheld and raw data were used for the analyses. For *Dactyloctenium*, stolon elongation data were skewed right for the T and TX treatment only. There was both significant heterogeneity of variance between groups, as well as an increase in variance with means. Rescaling of the data as well as a double log transformation (i.e. X = ln {ln (x + 1 + minimum)}) created the most normal distributions, and eliminated the increase of variance with mean. However, no transformation could produce homogeneity of variance between groups. Data were analysed with the above transformation anyway, as this is not a particularly serious violation of the ANOVA assumptions (StatSoft 2001).

When the experiment was designed, it was assumed that marked stolons would take root as they grew down the strip between the root trenches, and thus experience the benefit of the trenching despite the fact that their parent crowns were located right at the end of the trenches. Unfortunately this was often not the case, as many marked stolons did not produce rooted nodes as they grew. This experimental error may not have been particularly serious in terms of eliminating AG competition – in the TX treatments, the marked stolons would not necessarily have to take root to benefit from decreased shading. However, in terms of eliminating BG competition, it is may have been very serious – non-rooted stolons would rely entirely on parent crowns for the supply of water and nutrients, and these crowns may have experienced root competition for bunch grass around the trenches. In an attempt to control for this error, the above analyses were re-run excluding all data for intervals for which a given marked stolon did not have any rooted nodes.

For the landscape patterns experiment, stolon elongation data for *Digitaria* were approximately normal for all sites and the ANOVA assumptions of normality were not violated. For *Dactyloctenium*, data distributions were approximately normal, although some sites were skewed right. Data were therefore re-scaled and root transformed (i.e. X = root {x + minimum}). This eliminated an increase in variance with group means, but did not eliminate significant heterogeneity of variance between groups.

Rooted node production

Data for 'rooted nodes / stolon length' were highly skewed, usually with modes equal to zero and long tails. No transformation could produce normality, precluding the use of a MLM repeated-measures ANOVA as above. Again, as neither a GLZ ANOVA model, nor the Kruskal-Wallis non-parametric test are appropriate for repeated-measures analyses, the effect of treatments on the 'rooted nodes / stolon length' was not tested statistically.

Stolon survival

Standard survival analysis (also called failure-time analysis) was used to test for differences in stolon longevity, using the Survival Analysis module of Statistica v6.0. Although estimating the longevity of a stolon involves repeated observations, repeated-measures ANOVA analysis as above is not appropriate. This is because survival time data are usually strongly skewed and the mean and variance do not describe the data well (Fox 1993). Furthermore, ANOVA does not allow for the inclusion of censored data. In the case of this design, censored data involved only right censored data, i.e. those stolons which were not observed to die during the study period, either because their markers went missing or they were 'un-tagged' when they flowered. Although the longevity of these stolons is unknown, they still provide useful information in the form of a 'minimum' estimate of longevity (Fox 1993).

To account for the above features of survival time data, graphical analysis is usually done in the form of life table analysis, and significance testing by means of fitting non-linear distributions (Fox 1993; StatSoft 2001). For life table analysis the length of the study period is divided into equal intervals. For each interval the effective population size, as well as the probability of an individual dying in that interval, is calculated (Fox 1993, provides all formulas). Of all the variables which can be calculated thereafter (on an interval by interval basis), the most useful for comparison is the estimated cumulative survival probability, which is the fraction of individuals which has not died by the start of a given interval. An interval of 28 days was chosen for the life table analysis of the stolon survival data, as this generally incorporated two observations.

To test for the significance of observed differences in the cumulative distribution of survival times, one must choose between accelerated failure time models or proportional hazard models (Fox 1993). The former assumes that treatments and covariates (including time) affect the fraction of individuals surviving multiplicatively (i.e. a treatment would cause the chance of dying to increase for each interval). The latter assumes that chances of dying will only be increased by a treatment, for certain times of 'hazard' (i.e. for certain intervals only). For the analysis of stolon longevity, the proportional hazard model seemed appropriate, as it was expected that stolon death would occur during summer droughts, and that these would occur at the same time across all treatments (or sites), but would be made more severe by certain treatments (or at certain sites, such as the lowest altitude sites). Therefore, the Cox proportional hazards model was used. Incidentally, further analysis found that the use of an accelerated failure-time model (specifically a linear hazard model) produced the same results. In all these models, 'date of first measurement' was included as a covariate, as it was observed that stolon mortality appeared to be increase towards the end of the growing season. Statistica uses the maximum likelihood procedure to estimate the parameters for these models, and the goodness of fit is assessed using the Chi-squared distribution (StatSoft 2001).

RESULTS

1. Trenching experiment

Treatment effects

Trenching did reduce end-of-season root densities **(Fig. 4.4A)** but differences were only significant for *Digitaria* (students T test: *Digitaria*: $t_{17} = 2.43$, $p = 0.026$; *Dactyloctenium*: $t_{20} = 1.16$, $p = 0.26$). At the *Digitaria* site, the clearing of bunch grasses alongside the trenches worked as intended and the TX treatment had far lower average bunch grass heights in the 15cm strip alongside trenches. However, the T treatments also reduced bunch grass heights, relative to controls, which was not an intended effect **(Fig. 4.4B)**. The GLZ nested ANOVA model indicated that while differences between the controls and T treatment mean heights were highly significant, the T and TX mean heights were not significantly differently **(Table 4.2)**. At the *Dactyloctenium* site, bunch grass heights were higher, and clearing had not achieved the intended effect **(Fig. 4.4B)**. The GLZ nested ANOVA model indicated bunch grass heights in the TX treatment were not significantly lower than for the controls, although heights were significantly higher in the T treatment **(Table 4.2)**.

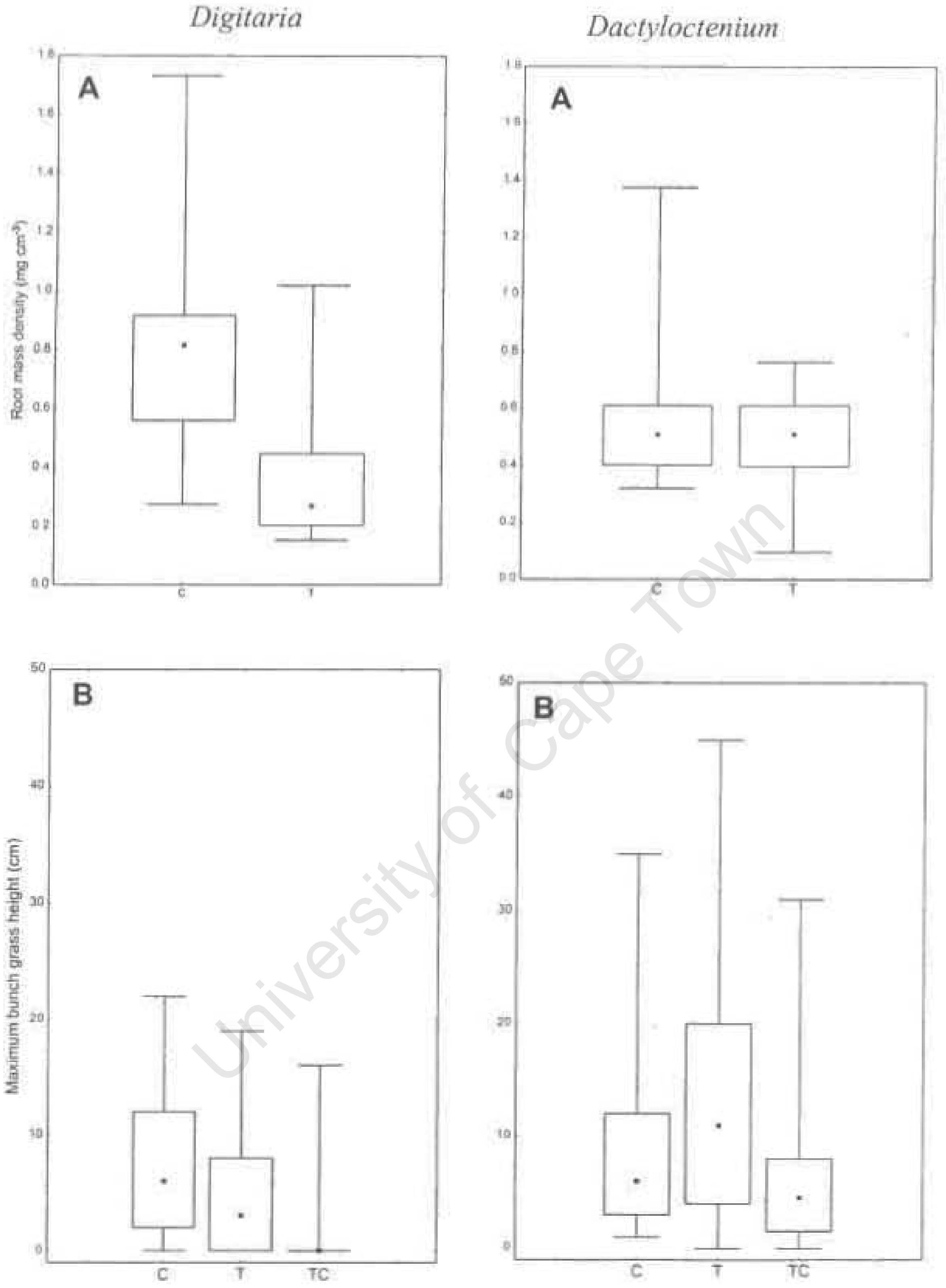


Figure 4.4. Trenching experiment. Treatments effects created for stolons of *Digitaria* and *Dactylocteium*. **A.** Median root mass density taken with 10cm root cores, outside (control) and inside trenches. Differences between treatments were significant for *Digitaria* only (Student t-test). **B.** Median bunch grass heights alongside stolons. Differences were significant for both species (see text for details). Treatments: C = control, T = trenching only, TX = trenching and clearing. Boxes show the 25th and 75th percentiles, whiskers show ranges.

Table 4.2. Trenching experiment. Parameter estimates from the GLZ ANOVA of maximum bunch grass heights measured alongside marked stolons in the various treatments at the Mbombe High 1(for *Digitaria*) and Mbombe (*Dactyloctenium*) sites. Models had a gamma distribution function and an identity link function. Note that the model was over-parameterized, i.e. the two treatments shown were tested against the 'trench + clear' treatment.

	Treatment	Estimate	Standard error	Wald	p
Digitaria					
Intercept		5.1	0.32	250	< 0.001
Treatment	Control	3.0	0.58	28	< 0.001
	Trench only	0.068	0.41	0.027	0.87
Scale parameter		1.3	0.11	147	< 0.001
Dactyloctenium					
Intercept		2.3	0.050	2126	< 0.001
Treatment	Control	-0.029	0.071	0.16	0.69
	Trench only	0.34	0.071	23	< 0.001
Scale parameter		1.52	0.12	157	< 0.001

Growth variables

Flowering

The proportion of marked stolons which flowered was not found to differ significantly between treatments (for *Digitaria*: $\Pi^2 = 0.36$, $p = 0.83$; for *Dactyloctenium*: $\Pi^2 = 0.74$, $p = 0.46$). Flowering appeared to decrease slightly with trenching for *Digitaria*, but was highest for the TX treatment for *Dactyloctenium* (**Fig. 4.5A**).

Final rooted nodes

The Chi-squared analysis revealed no significant treatment effects for the proportion of marked stolons which had rooted nodes at the time of last measurement (for *Digitaria*: *Digitaria*: $\Pi^2 = 1.54$, $p = 0.46$; for *Dactyloctenium*: $\Pi^2 = 2.07$, $p = 0.35$). However, values were twice as high with trenching for *Digitaria* (**Fig. 4.5B**). For *Dactyloctenium*, the reverse was true, with a slight decrease in final rooted node frequency observed for both trenching treatments.

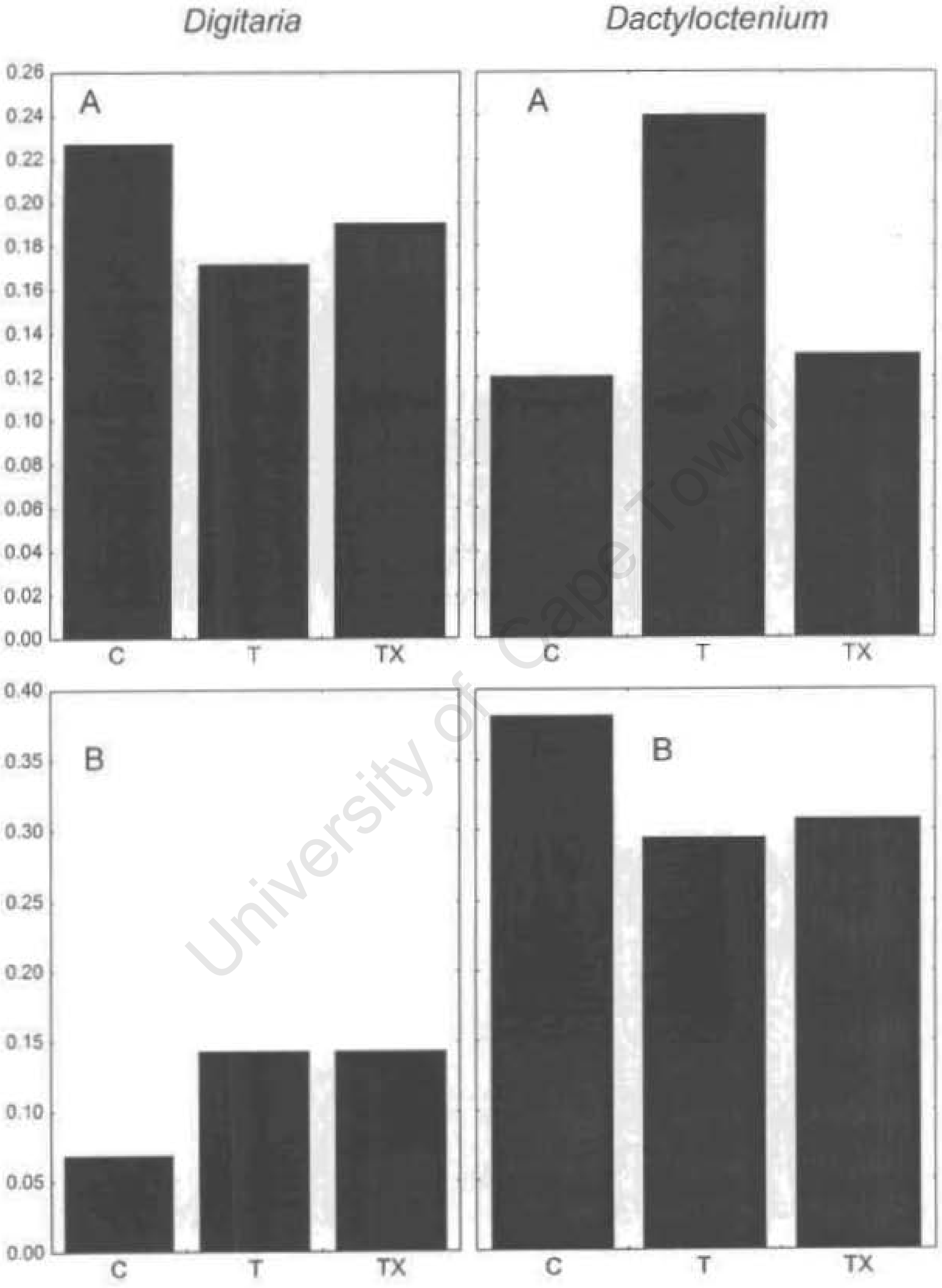


Figure 4.5. Trenching experiment. The proportion of marked *Digitaria* and *Dactyloctenium* stolons which flowered during the period of measurement (**A**), or had rooted nodes at the time of last measurement (**B**), for the control (C), trench (T) and 'trench and clear' (TX) treatments. Differences were not significant for either species.

Shoot production

Rates of shoot production (within intervals) were similar across all treatments (**Fig. 4.6A**). As a result, the MLM repeated-measures ANOVA's did not provide significant fits to the data (for *Digitaria*: $F_{132,219} = 0.70$, $p = 0.99$; for *Dactyloctenium*: $F_{134,234} = 0.94$, $p = 0.65$). Note that on the box and whisker plots, the confidence interval is far wider than one standard deviation, indicating the strong kurtosis of the data. Shoot production was close to zero for most intervals. However during certain intervals crowns would produce a burst of new shoots, only to lose them all at a later interval, resulting in the wide 'tails' in the data. This was particularly evident for *Digitaria*. At first this was thought to be in response to rainfall but neither the variance nor skewednesss of data within an interval appeared to be related to the rainfall of the interval (compare **Fig. 4.6A** and **Fig. 4.6D**).

Stolon growth

Stolon growth was far more variable between treatments and the MLM repeated-measures ANOVA's did provide significant fits to the data (**Table 4.3**). However, for *Digitaria* there was no significant effect of treatment, nor the 'treatment x interval' interaction. Despite this, **Fig. 4.6B** suggests that some treatment effects did occur in the second half of the growing season - for intervals 10 to 14, median rates of elongation (within an interval) were consistently higher for the TX treatment. This was after most of the rain for the season had fallen (**Fig. 4.6D**), and when grazing intensity was higher (pers. obs.)

For *Dactyloctenium*, the treatment effect was far stronger, although not significantly so at the 0.05 level (**Table 4.3**). Again there were a number of intervals where elongation was consistently faster for the trenching treatments (intervals 4 to 7 - **Fig. 4.6B**). These were all fairly wet intervals (**Fig. 4.6D**), and it was during these intervals that bunch grasses on the *Dactyloctenium* lawns were hardly grazed and began to grow tall.

Excluding all stolons without rooted nodes did not prove to be informative. For *Digitaria*, 83% of the data had to be excluded, and the resulting MLM ANOVA model did not provide a significant fit to the data ($p = 0.23$). *Dactyloctenium* is far more efficient in terms of establishing rooted nodes and only 44% of the data were excluded. However, the resulting model gave a very similar result (**Table 4.3**).

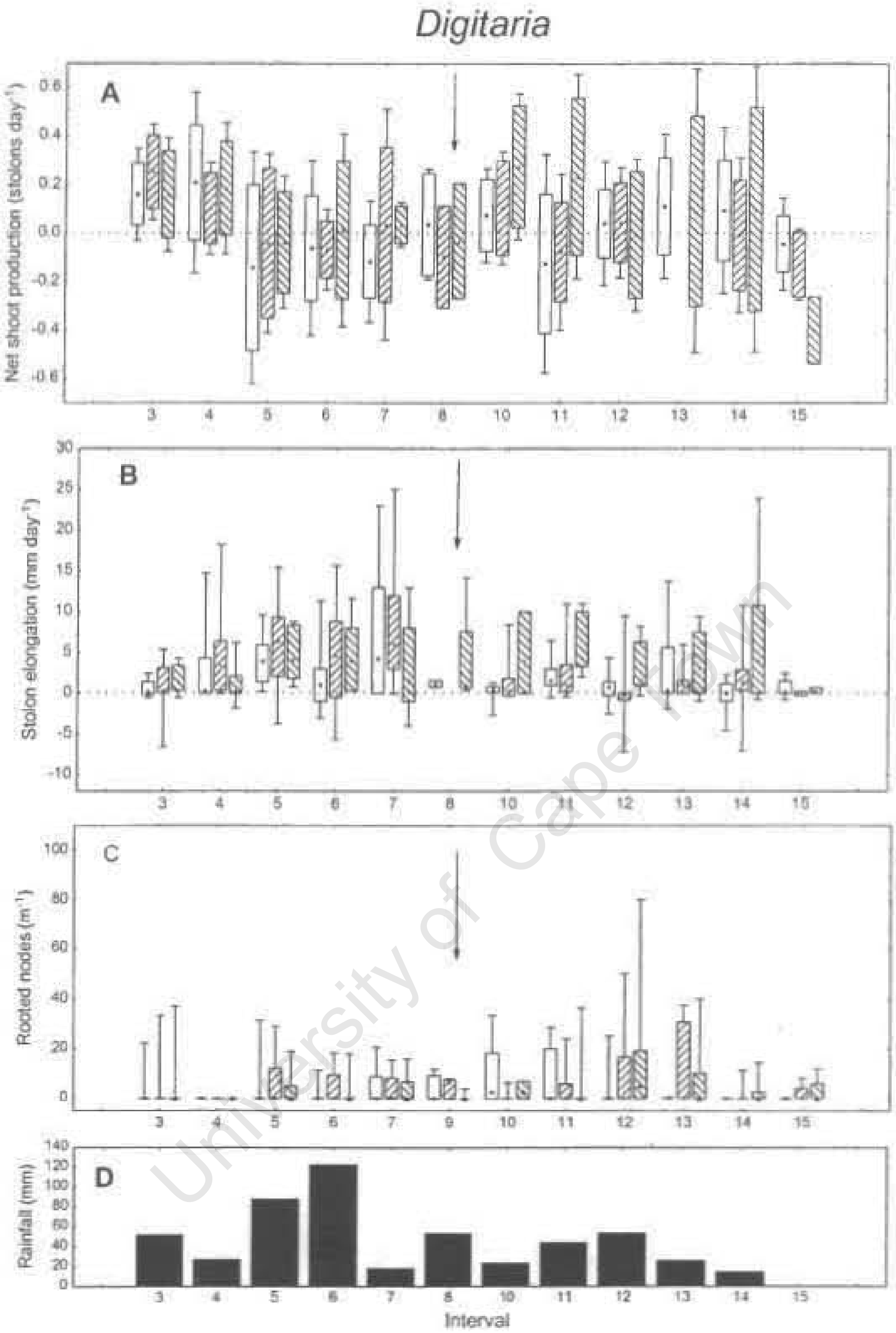


Figure 4.6. Trenching experiment. Stolon growth responses for each measurement interval (intervals durations range between 8 and 21 days), for the control (☐), trench (▨) and trench and clear (▨) treatments. **A.** Means of average rate of shoot production. Boxes show the 95% confidence limits, whiskers show one standard deviation. **B.** Medians of average rate of stolon elongation. Boxes show the 25th to 75th percentiles, whiskers show ranges. **C.** Median number of rooted nodes per stolon length. Boxes show the 25th to 75th percentiles, whiskers show ranges. No significant differences were found for any of the variables. **D** Rainfall for each interval, as recorded at a nearby weather station. Arrows indicates the time of mowing.

Dactyloctenium

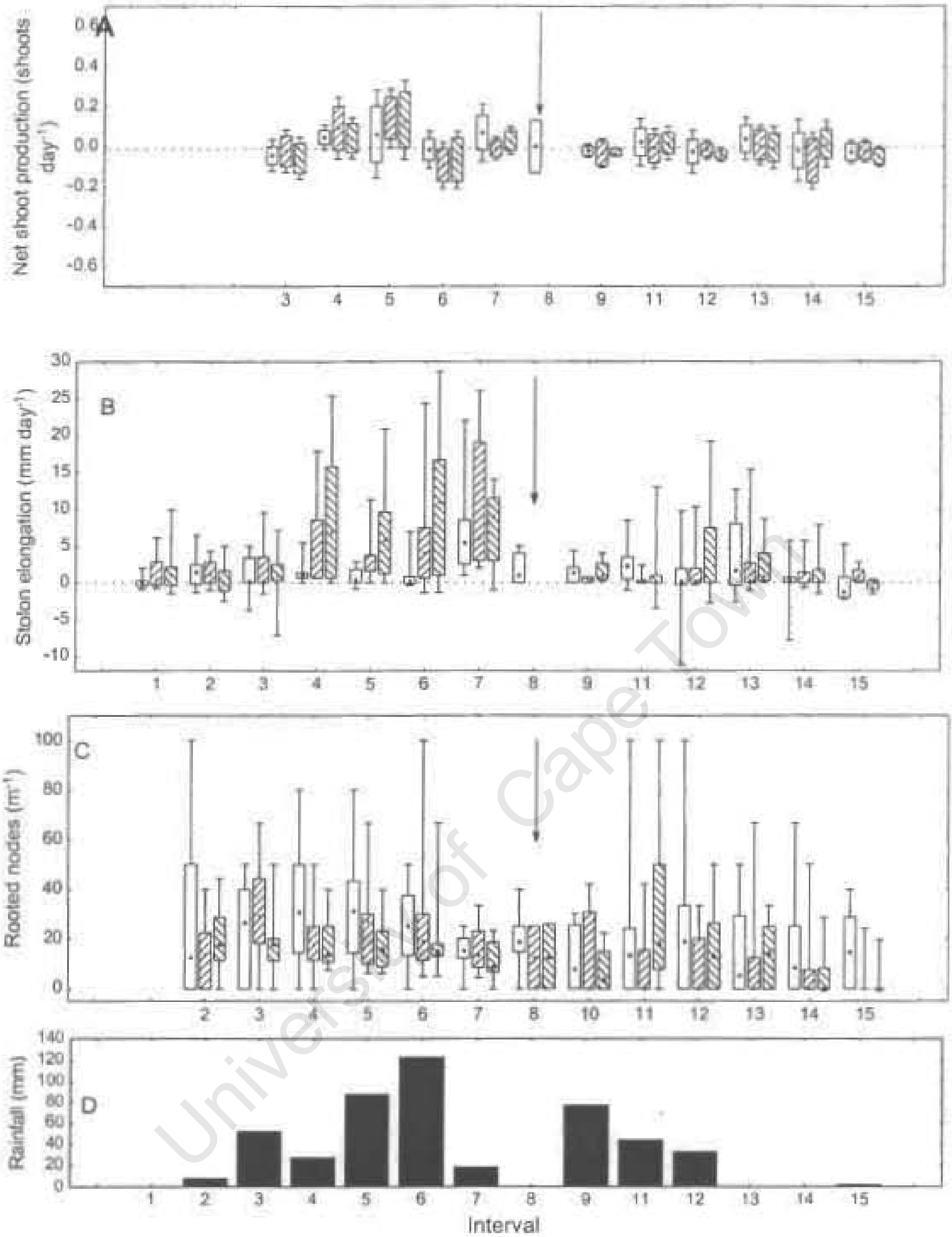


Figure 4.6. Continued

All the analyses showed a very strong effect of interval, i.e. averaged across all treatments, rates differed significantly between intervals. However, there were no significant correlations between either shoot production and rainfall or stolon elongation and rainfall for either species.

Table 4.3. Trenching experiment. Mixed Linear Model repeated-measures ANOVA analysis for average rates of stolon elongation (within intervals) for marked stolons of *Digitaria* and *Dactyloctenium*. For all analyses, data for which stolons had been bitten were excluded. df = degrees of freedom, MS = mean sum of squares.

	Effect	df	MS	F	p
Digitaria, all data: $F_{123,172} = 1.75$, $p < 0.001$					
Treatment	Fixed	2	5.9	0.27	0.76
Interval	Fixed	11	79	4.6	< 0.001
Treatment x interval	Fixed	21	15	0.86	0.65
Error		172	17		
Dactyloctenium, all data: $F_{133,228} = 2.36$, $p < 0.001$					
Treatment	Fixed	2	0.021	2.5	0.084
Interval	Fixed	13	0.045	6.5	< 0.001
Treatment x interval	Fixed	25	0.0091	1.3	0.16
Error		228	0.0070		
Dactyloctenium, stolons with rooted nodes only: $F_{90,111} = 3.57$, $p < 0.001$					
Treatment	Fixed	2	0.031	2.5	0.089
Interval	Fixed	12	0.028	4.6	< 0.001
Treatment x interval	Fixed	19	0.0075	1.2	0.28
Error		111	0.0063		

Rooted node production

While differences in the number of rooted nodes / stolon length were not tested statistically, **Fig. 4.6C** indicates that no consistent effects were created by the treatments. Again, no relationship between this variable and rainfall was evident.

Stolon longevity

Stolon survival times did not differ much between treatments, and cumulative survival curves showed overlap for both species (**Fig. 4.7**). For *Digitaria*, the mortality of stolons under approximately 100 days old appeared to be slightly lower for the trenching treatments. However, the Cox proportional models indicated no significant difference between treatments, for either species (**Table 4.4**). Date of first measure ('birth date') did influence survival times – the significant, positive parameter estimated for this variable indicating that stolons produced later in the season had greater longevity (for both species). This may have simply been an artefact of the shorter measurement period for stolons produced later in the season.

Table 4.4. Trenching experiment. Summary of the Cox proportional hazard models for *Digitaria* and *Dactyloctenium* stolon survival times. Π^2 values provide a measure of the goodness of fit of the models. Both the t-value and Wald statistic are shown for each independent variable. 'Birth date' refers to the first date of measurement, when stolons where only a few centimetres long.

	Parameter estimate	Standard error	t-value	Wald	p
Digitaria: $\Pi^2 = 30.3$, df = 2, $p < 0.01$, n = 118 (62 right censored)					
Treatment	-0.087	0.16	-0.53	0.29	0.59
Birth date	0.016	0.0033	4.9	24	< 0.01
Dactyloctenium: $\Pi^2 = 34.8$, df = 2, $p < 0.01$, n = 112 (66 right censored)					
Treatment	0.14	0.19	0.72	0.52	0.47
Birth date	0.018	0.0037	5.0	25	< 0.01

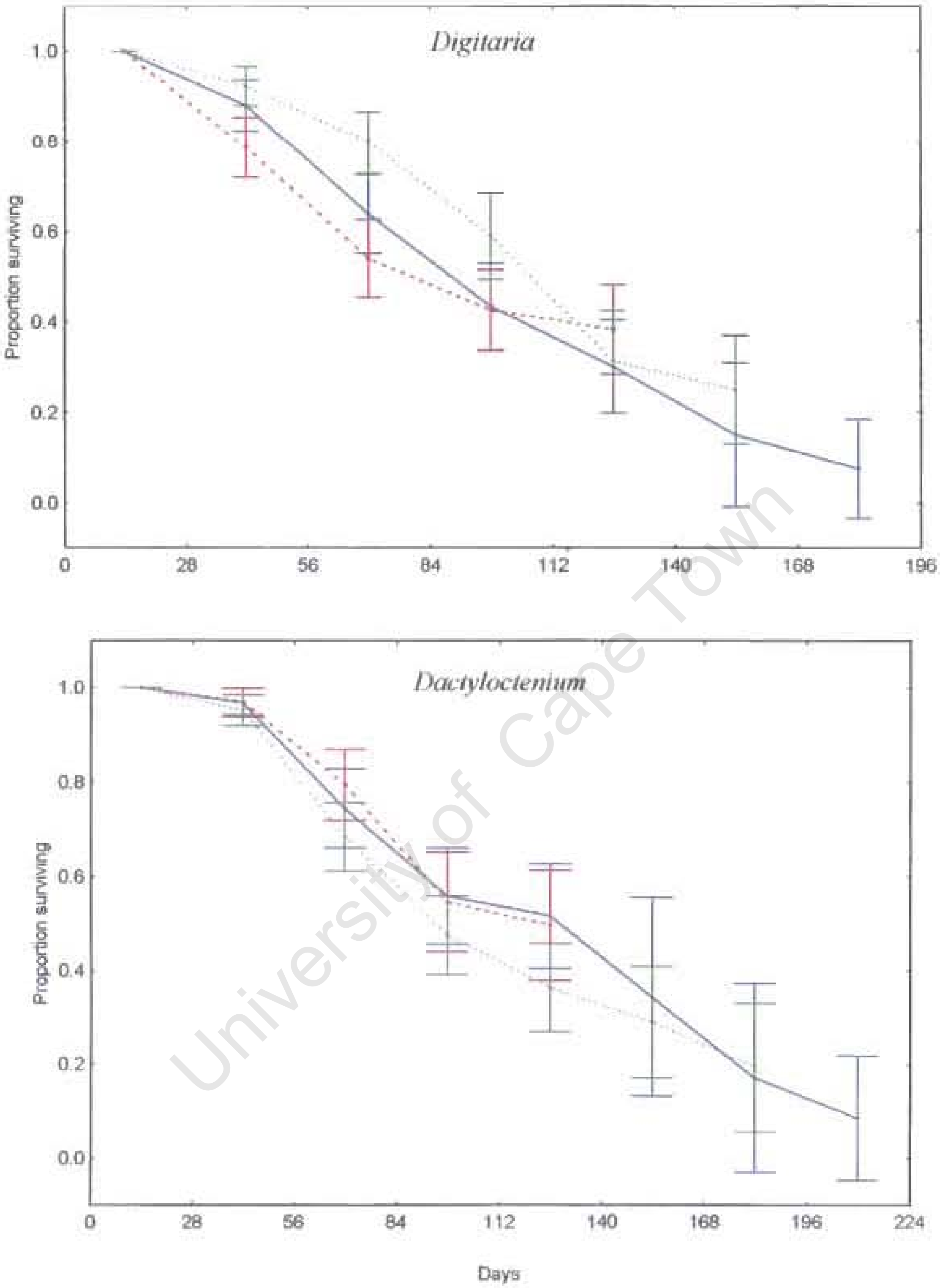


Figure 4.7. Trenching experiment. Stolon survival curves for marked lawn grass stolons of the control (···), trench (—)and 'trench and clear' (- -) treatments. Whiskers show standard errors.

2. Landscape patterns

Site differences

Table 4.1 summarizes differences in rainfall, mean distance to bunch grasses, and grazing intensity (as estimated by bitten stolon proportions) for the sites for which these were recorded. There were no statistically significant differences for the estimate of grazing frequency across all intervals (Kruskal Wallis test; for *Digitaria*: H = 7.6, n = 105, p = 0.28; for *Dactyloctenium*: H = 7.5, n = 104, p = 0.27). However, for any given interval the frequency of stolons bitten could vary greatly from site to site, an effect obscured by not using a repeated-measures analysis. Thus there is the potential error that grazing was confounded with respect to other environmental variables, e.g. during an interval when all sites experienced high rainfall, only some may have experienced heavy (or low) grazing as well.

Distances to bunch grasses could be analysed by more powerful parametric means, thanks to larger sample sizes. Nested ANOVA showed a strong effect of site for both species (**Table 4.5**). Generally, the distance to bunch grasses decreased with decreasing altitude, and this effect appeared to be greater for the *Digitaria* sites. (**Table 4.1**).

Table 4.5. Landscape patterns. GLM nested ANOVA of distance to bunch grass data for *Digitaria* and *Dactyloctenium* at the various sites at which stolons were marked. The eight nearest bunch grasses to each stolon were measured, and these data nested within sites.

	Effect	df	MS	F	p
Digitaria longiflora: $F_{74,544} = 25, p < 0.01$					
Intercept	Fixed	1	19853	3706	< 0.001
Site	Fixed	6	453	85	< 0.001
Stolon (Site)	Random	70	5.35	3.2	< 0.001
Error		544	1.66		
Dactyloctenium australe: $F_{96,649} = 7.4, p < 0.01$					
Intercept	Fixed	1	22990	5731	< 0.001
Site	Fixed	7	124	30	< 0.001
Stolon (Site)	Random	89	4.2	2.3	< 0.001
Error		649	1.9		

Root mass density differed little between the two high altitude lawn sites (Mbombe and Mbombe High 1) and one of the low altitude lawn sites (Nombali 2, **Fig. 4.8**). While root mass density was higher, in terms of medians and maxima for the adjacent ungrazed at the high altitude sites, mean differences were not significant (**Table 4.6**). At Nombali 1, the ungrazed bunch grass root mass densities were no different than on the lawn, but were significantly lower than those for the ungrazed swards at the higher sites.

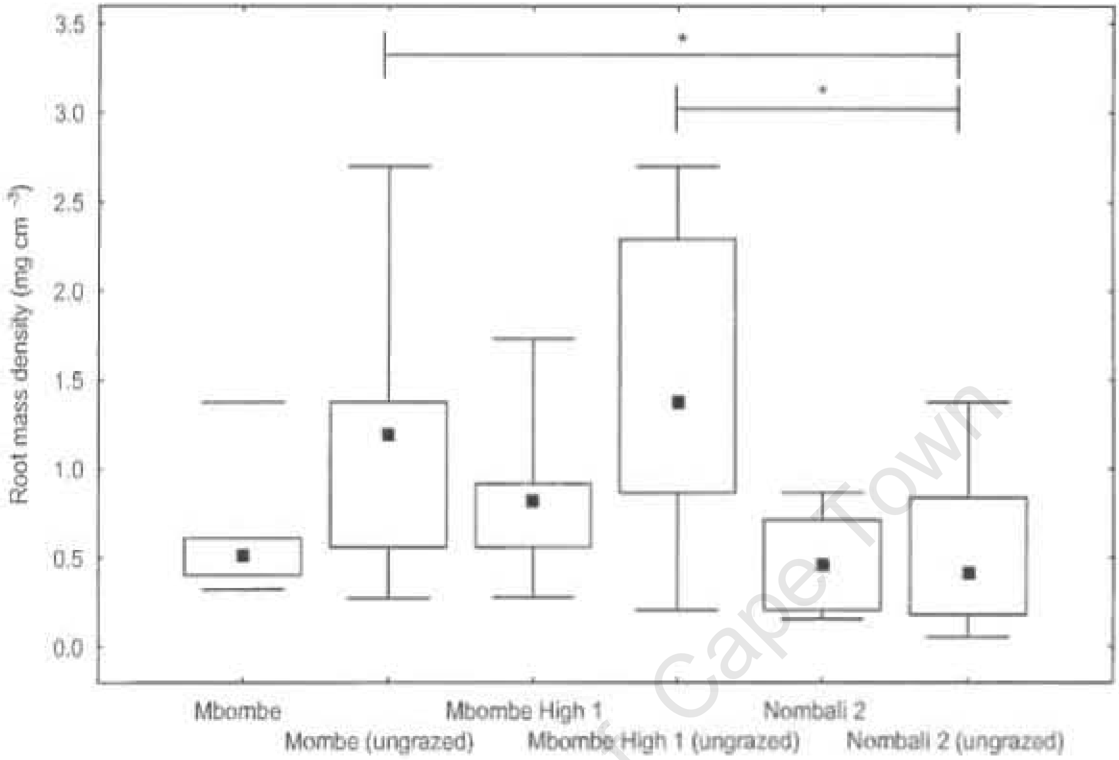


Figure 4.8. Landscape patterns. Median root mass density, taken with 10cm root cores at: the high altitude *Dactyloctenium* site (Mbombe), adjacent bunch grass swards at Mbombe, two high altitude *Digitaria* sites (Mbombe High 1and adjacent ungrazed site), and two low altitude *Digitaria* sites (Nombali 2 and adjacent ungrazed, bunch grass site). For the trenching experiment sites (Mbombe and Mbombe-High 1) data are from outside trenches only. Boxes show the 25th and 75th percentiles, and whiskers the range. *: pairs of sites significantly different ($p < 0.05$) according to a HSD post-hoc analysis of an ANOVA using ln transformed data.

Table 4.6. Landscape patterns. GLM ANOVA of ln transformed root mass density data. Samples were taken with 10cm root cores from lawn grass sites and adjacent swards of ungrazed bunch grass, at two high altitude and one low altitude site (see **Fig. 7** for post-hoc comparisons).

	df	MS	F	p
Site	5	2.8	5.0	< 0.001
Error	54	0.55		

Growth variables

Reproduction

Sites varied greatly in terms of the proportion of marked stolons which produced an inflorescence, but only significantly so for *Digitaria* (Chi-squared analysis; *Digitaria*: $\Pi^2 = 17$, $p = 0.008$; *Dactyloctenium*: $\Pi^2 = 8.84$, $p = 0.26$). For *Digitaria*, a greater proportion of stolons flowered at the higher altitude sites, while for *Dactyloctenium* there was no trend with altitude (**Fig. 4.9A**). However, for both species flowering was absent or minimal at the sites located in the drier part of HUP (Nombali 1, Nombali 2, Crossroads, Dunngamizi).

The proportion of stolons which had at least one rooted node at the time of the last measurement showed a more consistent pattern for *Dactyloctenium*, which produced far more stolons with rooted nodes than *Digitaria* (**Fig. 4.9B**). Chi-squared analyses indicated that between sites, differences were not significant for either species (*Digitaria*: $\Pi^2 = 6.7$, $p = 0.35$; *Dactyloctenium*: $\Pi^2 = 3.0$, $p = 0.88$). There was no obvious effect of altitude, although for *Digitaria*, it was the control transplants at the highest site (Mbombe) which had the greatest proportion of stolons with rooted nodes.

Shoot production

Fig. 4.10A shows that, when averaged across intervals, there was little difference in rates of shoot production (within intervals) between sites, for either species, and the MLM repeated-measures ANOVA's did not provide significant fits to the data.

The variance in the data generally increased with decreasing altitude for *Digitaria*. This was because rates of shoot production at the low-altitude sites (Crossroads particularly) were very high during certain intervals, and very low (i.e. negative) during others. This indicates a more stable growth environment for *Digitaria* at higher altitude with slow but continuous growth (which appeared to be the case for *Dactyloctenium*, at all sites). However, overall mean rates were similar and close to zero for all sites. The pattern of more extreme growth at lower altitude was observed to occur in response to large rainfall events, at least at the Crossroads site, but again no significant correlations were found between shoot production and rainfall (per interval).

Stolon elongation

For *Digitaria*, the treatments had little effect on rates of stolon elongation, with large variance in the data, both between treatment groups and measurement intervals. As a result, the MLM

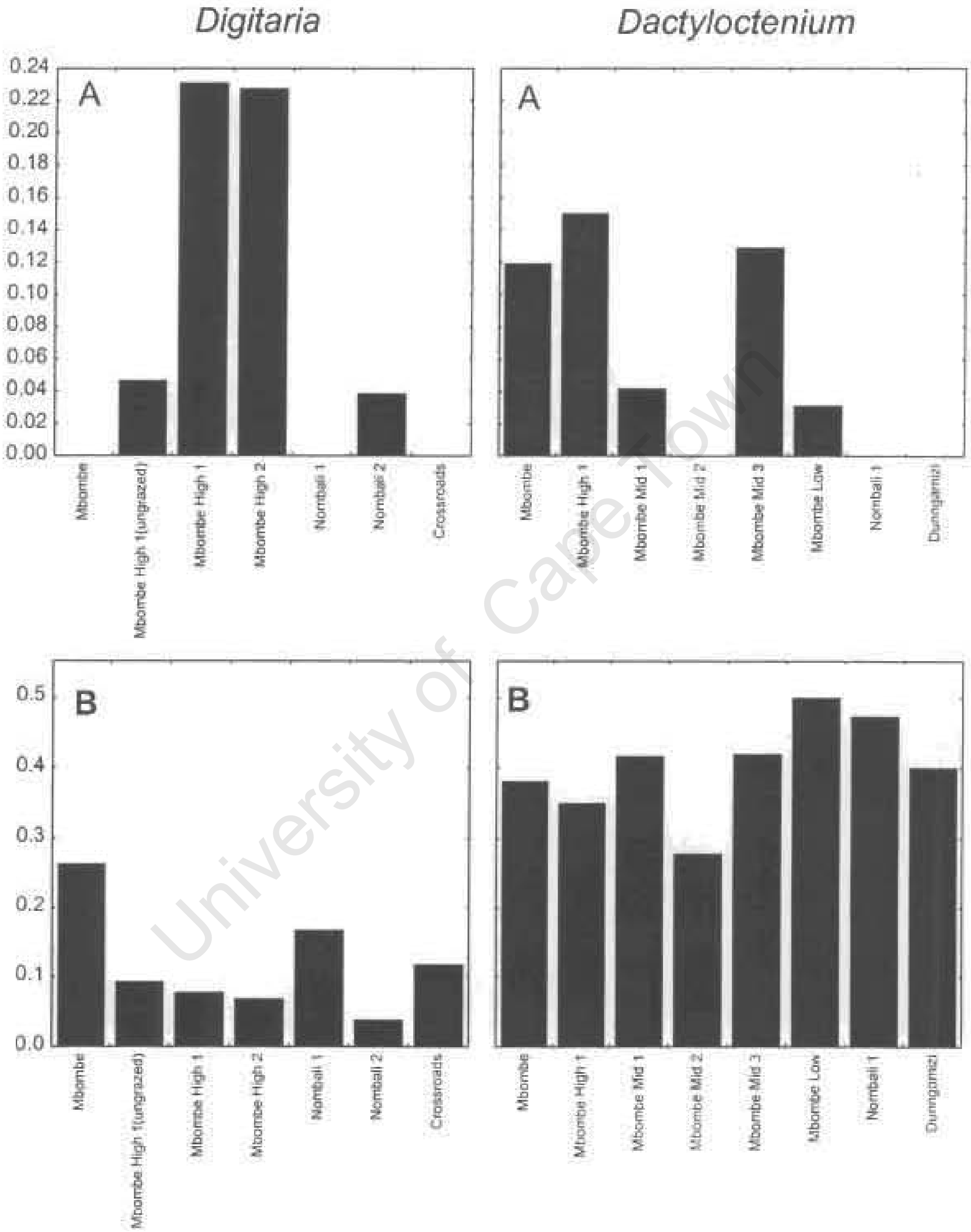


Figure 4.9. Landscape patterns. The proportion of marked stolons which flowered during the period of measurement (**A**), or had rooted nodes at the time of last measurement (**B**), for the various sites at which *Digitaria* and *Dactyloctenium* stolons were measured. In each graph, plots are arranged in order of descending altitude. Differences were only significant for the proportion of *Digitaria* stolons which produced rooted nodes (see text for details).

repeated-measures analysis of did not provide a significant fit to the data ($p = 0.10$). However means were clearly lower at the three low altitude sites (**Fig. 4.10B**).

Table 4.7. Landscape patterns. MLM repeated-measures ANOVA of average rates of stolon elongation (within intervals) for *Dactyloctenium* at eight sites in HUP. The model was significant ($F_{292,856} = 1.28$, $p = 0.005$).

	Effect	df	MS	F	p
Site	Fixed	7	0.14	1.7	0.10
Interval	Fixed	14	0.29	3.3	< 0.001
Site x Interval	Fixed	82	0.12	1.3	0.041
Error		856	0.09		

For *Dactyloctenium* variance was also high but seemed to decrease slightly with altitude (**Fig. 4.10B**). The relationship between means was more complicated, although the three highest altitude sites had greater means that the two lowest sites. The MLM repeated-measures ANOVA did provide a significant fit to the model, with the significant 'sites x interval' interaction indicating that differences between sites varied from interval to interval (**Table 4.7**).

Rooted node production

With the exception of those at Crossroads, *Digitaria* stolons had, on average, no rooted nodes (**Fig. 4.10C**). However, during some intervals some of the longer stolons did produce a few rooted nodes, hence the long positive tail in the data. In terms of maximum values, there did appear to be some differences between sites, but both high and low altitude sites had the highest maximum values. At the high altitude where the trenching experiment was conducted (Mbombe High 1) stolons of the control treatment produced the smallest number of rooted nodes per stolon length.

Dactyloctenium stolons took root more readily, and data were less skewed (**Fig 4.10C**). In addition, there appeared to be a divide between the lower and higher altitude sites - very high values (indicating that all or almost all nodes had rooted - pers. obs.) were recorded at the lowest altitude sites only.

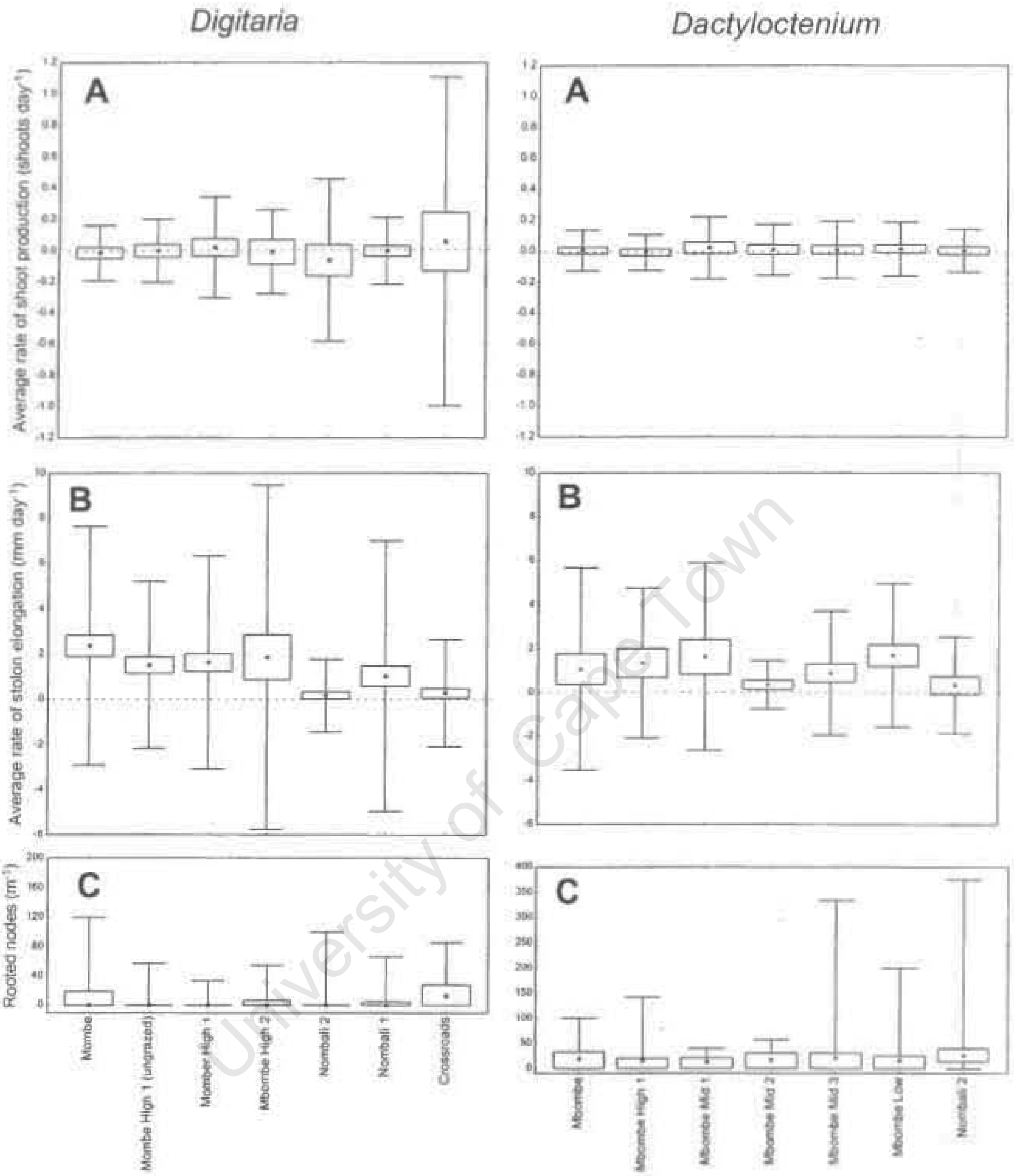


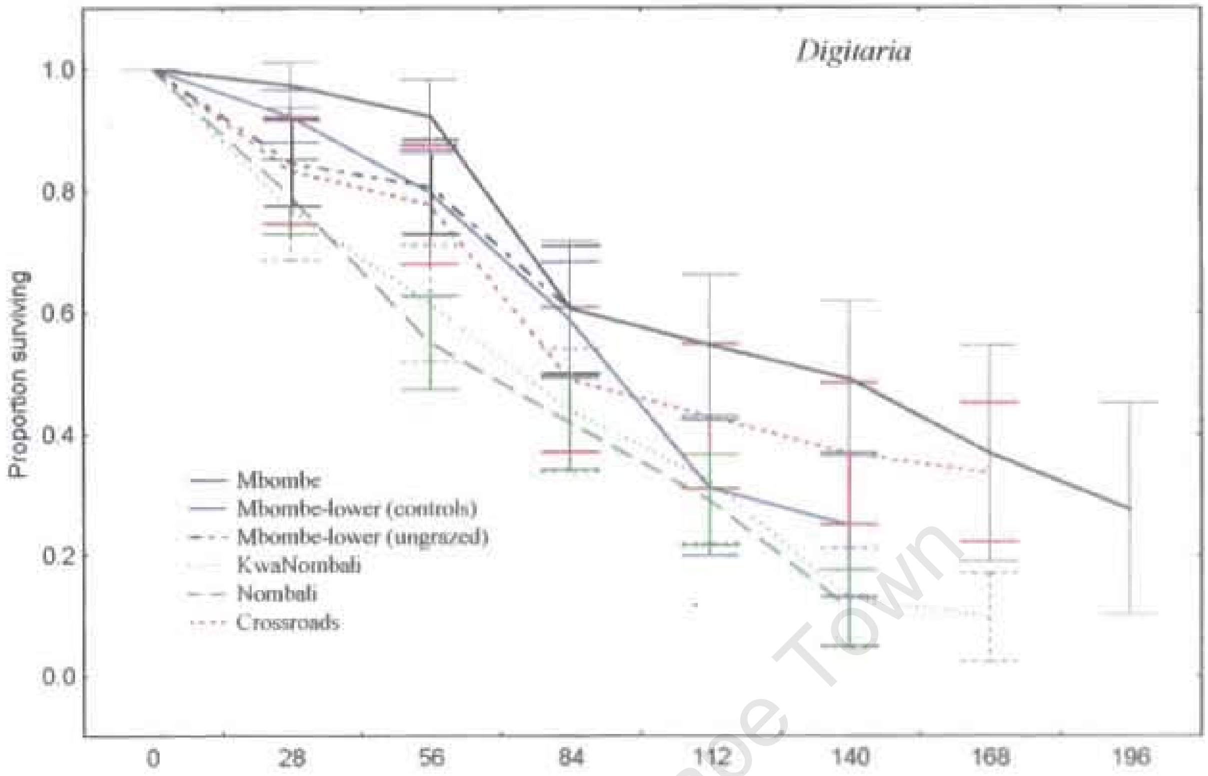
Figure 4.10. Landscape patterns. Stolon growth responses, averaged across intervals, for *Digitaria* and *Dactyloctenium* at each site in Hluhluwe-Umfolozi Park where they were measured. **A.** Means of average rates of shoot production within an interval. Boxes show the 95% confidence limits, whiskers show one standard deviation. **B.** Means of average rates of stolon elongation within an interval. Boxes show the 95% confidence limits, whiskers show one standard deviation. **C.** Median number of rooted nodes per stolon length in an interval. Boxes show the 25th to 75th percentiles, whiskers show ranges. Sites are arranged in order of descending altitude. Differences were only significant for average stolon elongation rates for *Dactyloctenium* (see **Table 4.7**).

Stolon longevity

As with the trenching experiment, stolon survival times were short with only a small proportion of the marked stolons surviving for the entire duration of the study period. Life table analysis revealed only slight differences between sites. For *Digitaria*, the cumulative survival plots show that stolons at two of the driest sites (Nombali 1 and Nombali 2) had the lowest survival times, while the those at the highest site (Mbombe) had the greatest longevity **(Fig. 4.11)**. However, the other high and low altitude sites (Mbombe High 1 and Crossroads) showed overlapping, intermediate values, and the Cox proportional hazards model indicated no significant differences in survival times between the various sites **(Table 4.8)**. Again 'birth date' had a very significant effect. The cumulative survival curves showed even greater overlap for *Dactyloctenium* **(Fig. 4.11)**, and the two sites with the shortest survival times were both the highest (Mbombe) and one of the lowest (Mbombe Mid 3). The Cox proportional hazards model showed no significant effect of site **(Table 4.8)**.

Table 4.8: Landscape patterns. Analysis of the Cox proportional hazard models for *Digitaria longiflora* and *Dactyloctenium australe* stolon survival times at various sites. Π^2 values provide a measure of the goodness of fit of the models. Both the t-value and Wald statistic are shown for each independent variable. 'Birth date' refers to the first date of measurement, when stolons were only a few cm's long.

	Parameter estimate	**Standard error**	**t-value**	**Wald**	**p**
Digitaria longiflora: $\Pi^2 = 34$, df = 2, $p < 0.001$, n = 192 (73 right censored)					
Site	-0.0008	0.004	-0.18	0.033	0.86
Birth date	0.012	0.002	5.6	31	< 0.001
Dactyloctenium australe: $\Pi^2 = 39$, df = 2, $p < 0.001$, n = 266 (154 right censored)					
Site	-0.072	0.047	-1.5	2.4	0.13
Birth date	0.014	0.0025	5.6	32	< 0.001



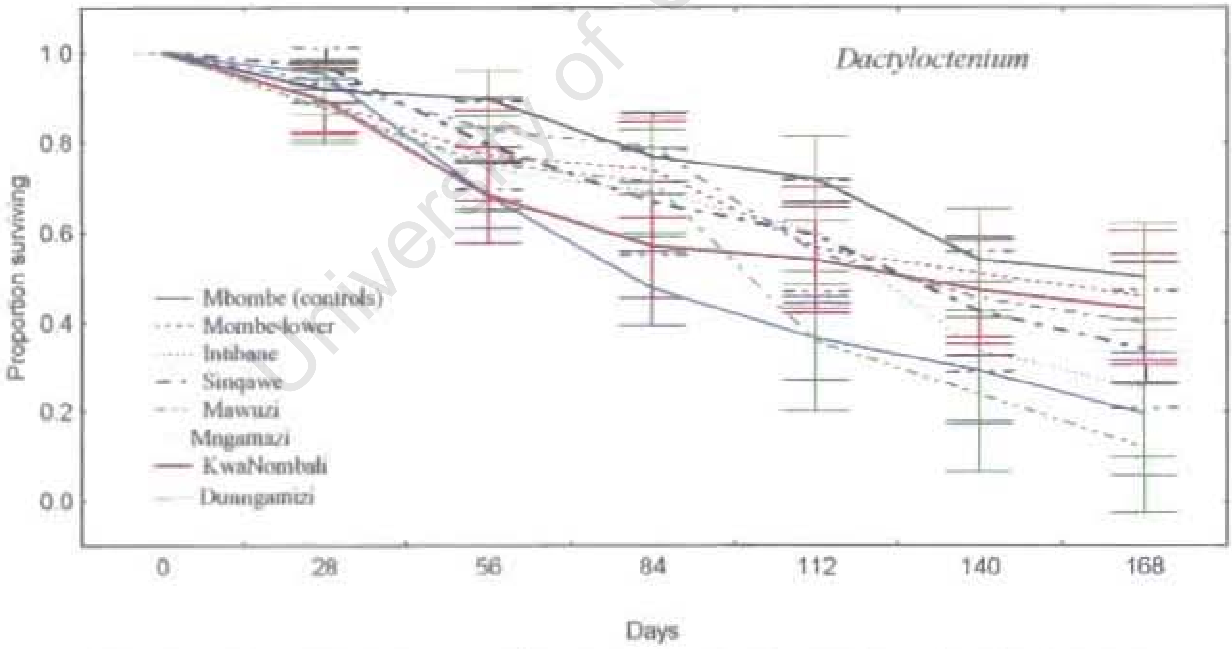


Figure 4.11. Landscape patterns. Stolon survival curves for marked stolons for the sites at which *Digitaria* and *Dactyloctenium stolons* were measured. In the legends, sites are arranged in order of descending altitude. Whiskers show standard errors.

DISCUSSION

1. Trenching experiment

The results of the trenching experiment provided little evidence that the growth of either *Digitaria* or *Dactyloctenium* is affected by competition from bunch grasses in a heavily-grazed, mesic environments of HUP. Other than the increase in the proportion of *Digitaria* stolons which rooted nodes at the end of the season (**Fig. 4.5**), and the slight increase in *Digitaria* stolon longevity (**Fig. 4.7**), there were no clear treatment effects. The former pattern, of greater nodal rooting, was probably a result of a lack of physical obstruction (above-ground) created by clearing away bunch grass crowns inside the trenches. Whether these increases would have translated into greater growth the following season (i.e. multi-season growth) is unknown, but the result does raise the possibility that competitive exclusion of lawn grasses occurs through the monopolization of space, a factor not considered thus far. Alternatively, it might indicate that BG competition from bunch grasses occurs through the inhibition of the establishment of young 'stolon roots', rather than lowering nutrient uptake by established 'crown roots'. Regardless, the similar rates of stolon elongation suggest that this form of competition is of little importance.

Potential experimental errors

The lack of consistent differences reported for the remaining growth variables measured needs to be considered in the context of potential sources of experimental error. The high variance in the data may indicate experimental error, and the method of trenching employed to eliminate BG competition may have not worked effectively. Alternatively, high variance in the data may simply reflect natural variation in the growth of the species measured, particularly as this was found for a much larger sample of plants in the 'landscape patterns' experiment. The use of open-ended trenches to exclude BG competition from neighbours needs careful consideration, as no reports of the successful use of such a technique were found in the literature. While some of the marked stolons of the T and TX treatments did elongate at rates comparable to those in plots completely cleared of bunch grasses (herbicide experiment, chapter 3), with elongation rates of over 2cm per day, this did not occur consistently (**Fig. 4.5B**). A potential explanation for this is that the trenches limited the growth of many of the marked stolons by restricting rooting volumes. However, this is unlikely as firstly, the volume of soil within the trenches was far greater than the rooting volume which the rooted stolon nodes are likely to have produced in a single growing season. Secondly, those marked stolons which did elongate far faster than in the cleared plots generally produced more rooted nodes and presumably a greater density of roots within the trenches. A more likely explanation is that that trenching failed to completely eliminate either AG or both BG and AG competition

AG versus BG effects

The grass height measurements indicated that the elimination of AG competition was not achieved as intended, particularly for *Dactyloctenium*. If shading alone was responsible for the lack of large growth responses, one would expect the greatest effects of the TX treatment at times when surrounding bunch grasses were tallest (before the mowing in the middle of the season), and the smallest effects when bunch grasses were short and shading minimal (at the beginning of the experiment, and immediately after the mowing). For *Dactyloctenium* this appears to be the case, as rates of stolon elongation were consistently higher for intervals 4 to 6 (**Fig. 4.6B**). However growth during intervals when bunch grasses were short was lower, and similar across all treatments, indicating BG competition for all treatments. Differences in rainfall cannot explain this lack of treatment effects as growth on the TX was very high during interval 4 which had low rainfall (**Fig. 4.6**), but low during interval 9 with high rainfall.

For *Digitaria*, growth on the TX treatment was not consistently greater than the C and T treatments in the first half of the season (before the mowing) which suggests that BG competition prevented the transplants from responding to any increases in light supply created by the clearing treatment. When growth on the TX was greater, in the second half of the season, it was hardly greater than that which had been achieved by controls earlier in the season, again indicating BG limitations to growth.

Trenching effectiveness

The location of the measured lawn grasses relative to the trenches may have resulted in overlap between lawn grass roots and those of neighbouring bunch grasses (**Fig. 4.3**). Whether this affected stolon elongation as well depends on whether bunch grass roots occurred all the way down the inside of pairs of the trenches as well, and on the degree of physiological integration between the lawn grass crowns and the marked stolons.

Root densities within pairs of trenches

The measure of root mass density probably overestimated BG competition, as it did not include only live bunch grass roots. Samples would have included some roots of the target lawn grasses, and for *Dactyloctenium* could have included roots from non-target stolons allowed to grow over the trenches as well. In addition, as live roots could not be extracted from the soil samples, some of the root mass recorded for the T and TX treatments may have consisted of dead bunch grass roots not yet decomposed. This could explain why reductions in root mass density created by trenching were quite small (and for *Dactyloctenium*, non-significant). Despite this, it is possible that a significant portion of the live root mass inside the trenches belonged to bunch grasses, and that this did constrain the growth of the marked stolons in some way.

Physiological integration

A high degree of physiological integration (in a basipetal direction) could have transferred some of the effects of the competition experienced by lawn grass crowns onto the marked stolons. However, as the exclusion of stolons without rooted nodes had very little effect on the results this seems unlikely. Furthermore, tracer ^{15}N experiments suggest that physiological integration is limited in grasses (Briske and Derner 1998), although few such studies have been performed and all involve caespitose species.

Other potential errors

In addition to the problem that the trenching treatments did not seem to eliminate competition from bunch grasses, as intended, there are two further experimental errors which should be considered.

Intraspecific competition

While the Mbombe site had the greatest density of bunch grasses (**Table 1**), the majority of the basal cover actually consisted of a dense lawn of *Dactyloctenium* stolons. Damage to many of these stolons was inevitable when the trenches were dug and the border strips cleared, and even though they were allowed to re-grow inside the trenches, the degree of intraspecific competition experienced by marked stolons inside the trenches must have been less than that experienced by the controls. Therefore, intraspecific competition may have constrained the growth responses of this species, particularly on the TX treatment. As intraspecific competition can be stronger than interspecific competition for grasses (Banyikwa 1988; Anderson and Briske 1995), this may have been a significant error.

Grazing

The suspected tendency of grazers to target stolons within the trenches may explain the slightly higher proportion of *Digitaria* control stolons which flowered and the lower mortality of *Digitaria* control stolons under 100 days old. For rates of stolon elongation, the elimination of bitten stolons from the analyses was intended to correct for any confounding effect of this selective grazing. However, if faster growing stolons were more likely to be grazed, then eliminating bitten stolons would have eliminated the fastest growing stolons from the trenching treatment data but not from the control data. A plot of the probability of a stolon being bitten (in an interval) against stolon length (at the start of the interval) suggested that this was not the case – although the probability of being bitten increased with stolon length, it did so in a similar fashion for each treatment. Therefore grazing, and the elimination of bitten stolons from the data set tested, cannot explanation the lack of consistently high stolon elongation rates reported for the trenching treatments.

Grazing may have also affected rates of nodal root production, as grazers can uproot nodes proximate to the point of the biting. McNaughton (1984) found that the density of rooted shoots of a population of the stoloniferous grass *Cynodon dactylon* was negatively related to grazing intensity, and in this study uprooted stolon nodes were often observed on the longer, bitten stolons. This may explain why *Dactyloctenium* stolons of the trenching treatments had fewer rooted nodes, per length of stolon, in many intervals (**Fig. 4.6C**).

2. Landscape patterns

Within-season growth

Regardless of whether one accepts the results of the trenching experiment, the comparison of the growth of the control stolons of that experiment with that of stolons from sites where grazing lawns are common, provided good evidence that lawn grass growth at the mesic extreme of HUP is not constrained by competition any more than at drier sites. The equal or greater rates of shoot production, stolon elongation, rooted node production and the similar stolon survival times all show that within-season growth was no lower at high altitude sites than at low altitude sites. Again this does not indicate an absence of bunch grass competition - growth at all sites was clearly less than that observed in the plots cleared of all bunch grasses in the herbicide experiment of the previous chapter. Rather it appears that any increases in competition at the higher sites are countered by changes in abiotic environmental conditions (such as higher rainfall). In fact, higher rainfall may even over-compensate for increased competition, as growth was under-estimated at the high altitude, mesic sites (Mbombe and Mbombe High). This under-estimation was a result of sampling not extending over the entire growing season. In the weeks following the final measurements, stolons at these sites were observed to continue growing as a result of subsequent rainfall, which did not occur at the low altitude sites.

On the contrary, one result which indicates reduced within-season growth at the higher rainfall sites is that of the rate of rooted node production for *Dactyloctenium*. Maximum rates were far greater at the low altitude sites (**Fig. 4.10C**). As with *Digitaria* in the trenching experiment, this may reflect a greater degree of AG physical obstruction created by a higher basal cover of bunch grass crowns. However, as these differences did not translate into differences in either rates of stolon elongation, or final proportions of rooted stolons, they are unlikely to be of much consequence.

Root mass density as a measure of BG competition

The higher basal cover of bunch grass at the high-altitude grazing lawn sites (**Table 1**) suggest that lawn grasses experienced greater (interspecific) competition at these sites, but this is not supported by the root mass density data. Rather, the root mass density data suggest that, with a similar intensity of grazing, bunch grass roots are reduced to the same density across the altitudinal gradient (**Fig. 4.8**). This in turn suggests that the root:shoot ratio of grazed bunch grasses is lower at higher altitude, which is plausible considering that the supply of soil water, and possibly other soil resources, increases with altitude.

The root mass density pattern also suggests that bunch grass competition does not increase with increasing rainfall, on the assumption that root mass density is a good surrogate for BG competition (i.e. assuming that rates of resource uptake per unit mass do not differ between sites). However, consideration must be given to the measure of root mass density when interpreting this pattern, as no distinction was made between lawn grass and bunch grass roots. As the total basal cover of the high and low altitude sites appeared similar, it is more likely that the root mass densities of bunch grasses were in fact lower at the low altitude site (Nombali 2), with lawn grass roots making up the difference. Therefore the original proposal that bunch grass competition increases with rainfall, for a given intensity of grazing, is not contradicted by the root mass density pattern. What the root measurements did contribute, is that the root mass density of lawn grasses (and presumably intraspecific BG competition) probably decreased with increasing rainfall. To summarize then, the most likely set of environmental conditions, besides a similar intensity of grazing, which produced the similar growth patterns over the period of measurement are:

- low rainfall, low interspecific competition (from bunch grasses) and high intra-specific competition at the low altitude sites;
- high rainfall, high interspecific competition and low intraspecific competition at the high altitude sites.

Multi-season growth

The results of the landscape experiment lead one to the conclusion that lawn grass growth is unaffected by any BG competition from bunch grasses, and that with a high and sustained level of grazing, grazing lawns should be as common at high altitude as they are in the lower altitude parts of HUP. However, these results are for a single season's growth only, and extrapolation to other years must be done with caution, for three reasons in particular. Firstly, vegetative reproduction was estimated rather crudely, and any differences which were overlooked could be important, particular as this form of reproduction is known to be important under conditions of heavy grazing (Belsky 1986b; O'Connor and Everson 1998). Secondly, storage of growth between growing seasons is also likely to be an important part of multi-season growth, but this was not measured. Thirdly, rainfall was particularly low during the experimental period, and results may have differed if the experiment was conducted in a different year.

Vegetative reproduction

While differences in the rate of nodal root production were small **(Fig. 4.10C)**, the particularly high maximum rates for *Dactyloctenium* may be of consequence in the long term. Although these high rates did not translate into any differences in the proportion of 'stolons with at least on rooted node at the last time of measure', the number of rooted nodes per stolon (at the last time of measure) may well have differed. Such a measure (i.e. the proportion of all nodes alive at the end of the growing season which were rooted) may have exposed differences in vegetative reproduction which the current measure did not. Furthermore, differences in the proportion of stolons with a rooted node between the control and trenching treatments of the trenching experiment were evident **(Fig. 4.5B)**, despite the potentially confounding effect of selective grazing. Therefore a potentially important long term effect of bunch grass competition – that of monopolizing AG space – may have been overlooked.

Storage

If the lawn grasses at the high altitude sites allocated relatively more assimilates to growth than storage, similar rates of within-season growth may have been achieved at the expense of reduced multi-season (long term) growth, particularly if future growing seasons brought less favourable environmental conditions. While allocation to storage was not measured, the higher maximum rates of shoot production for *Digitaria* at two of the drier sites **(Fig. 4.10A)** offers evidence that it may have differed. If the large 'pulses' of shoot production at the low altitude sites resulted in a proportionately greater net gain of assimilates during periods of high resource supply, then the potential for greater storage by plants at these sites exists.

Even if growth during such pulses was not allocated to storage, the fact that similar maximum rates of shoot production were not achieved at the higher altitude sites indicates a restriction on lawn grass growth there. Why would *Digitaria* plants in a more mesic environment not be able to achieve high rates of shoot production? Increased shading resulting from a higher density of bunch grasses is an unlikely reason. Firstly, the results of the trenching experiment indicate that the removal of all shading did not affect rates of shoot production. Secondly, when *Digitaria* does grow in deep shade, it does not appear to reduce rate of shoot production – instead shoots develop into short, vertical culms rather than stolons (pers. obs.). Abiotic constraints such as lower temperatures can also be ruled out on the basis of the rapid growth of the transplants in the herbicide experiment (chapter 3). Once again, some form of BG competition is the most likely explanation, although in this case the effects of the competition may only become clear after many growing seasons.

Rainfall

The growing season during which the experiments were conducted was a dry one, with total (summer) rainfall well below the average for both weather stations. During wetter years, additional competition resulting from any enhanced bunch grass growth could be higher at the mesic end of the gradient, considering the higher basal cover of bunch grasses there (Table 4.1). For example, if grazing intensity were constant, higher rainfall would lead to more rapid regrowth of bunch grasses between grazing events, leading to greater shading of lawn grasses.

In this case, potential greater growth of the lawn grasses would not counter the effects of this additional competition, as short, prostrate species *Digitaria* and *Dactyloctenium* could not compete with bunch grasses for light enough with an optimum supply of water.

The surprisingly good growth of *Digitaria* at the ungrazed high altitude site (Mbombe High 2 – ungrazed) suggests that even if an increase in bunch grass competition during a wet year was severe, it would not eliminate lawn grasses. However, a number of consecutive years of above-average rainfall might produce different results. If grazer populations increased as well under such circumstances, to the point that any potential increases in bunch grass growth were nullified by increases in grazing intensity, then rainfall might not be of much consequence to the multi-season growth of lawn grasses. However, it seems unlikely the growth of grazer populations could be fast enough to track changes in annual rainfall (which can be quite dramatic, even in the mesic parts of HUP – rainfall data, Hluhluwe Research Centre). Therefore, the possibility that the lawn grass growth would be reduced to the point of (competitive) exclusion during a spell of 'wet years' must be borne in mind.

Conclusion

The results of the trenching experiment provided no evidence that bunch grass competition excludes either *Digitaria* or *Dactyloctenium* from heavily grazed, high altitude areas of HUP. However, the experiment contained potential errors, particularly regarding the effectiveness of the root trenches used. Nevertheless, these results were substantiated by the landscape patterns experiment. The results of this experiment revealed very little difference in any of the components of within season-growth measured. However, extrapolation of this pattern to future growing seasons must be done with caution, as multi-season growth was not measured directly. Vegetative reproduction and storage could be particularly important in this regard, as observed patterns of nodal root production and shoot production could indicate differences in these growth components. In addition, results may have differed in growing seasons of above-average rainfall.

CHAPTER 5

SYNTHESIS

The experimental work described in the previous chapters does not provide support for the importance of the key ecological processes, other than frequent, non-selective grazing, proposed to be necessary for the formation and persistence of grazing lawns in chapter 1. This chapter considers the validity of these results, as well as their relevance to grass-grazer systems in general. Thereafter, other ecological processes which may explain the limited distribution of grazing lawns are considered. Specifically, the possibility that the distribution of grazing lawns is not constrained by any additional key processes, but rather by a multitude of species-specific factors, and the possibility that a lack of non-selective grazing is limiting in mesic systems are explored. Note that the species involved in the previous chapters are again referred to by their generic names in this chapter.

Experimental results

Nitrogen

The lack of a common preference for a particular source of nitrogen (N) (chapter 2 section 2), the differences in vegetative growth responses to N supply (chapter 2 section 3), the limited or lack of response to fertilization in the field (chapter 3), and the lack of response of HUP lawn grass species to root trenching (chapter 4) all indicate that a high supply of N is not a key process which limits the distribution of grazing lawns in HUP. Certain results indicate that N supply could exclude certain species: *Urochloa* showed a slight preference for the nitrate source of N in the sand culture experiment, and showed a strong response to N supply in the solution culture experiment, and *Dactyloctenium* responded to fairly small levels of N fertilization. However, these results cannot explain the absence of grazing lawn communities in general: even if a low supply of N restricts the distribution of *Urochloa* and *Dactyloctenium*, this is highly unlikely to exclude *Digitaria* and *Sporobolus* (from heavily grazed environments). Furthermore, the results of chapters 2 and 3 which indicate that *Dactyloctenium* could experience N limitations (a fairly strong response to N in solution culture, and to N fertilization in the field) were superseded by the results of chapter 4. The landscape patterns experiment described in that chapter indicated that with regular grazing, any competition for N does not reduce the growth of this species below that which occurs in the drier parts of HUP, where *Dactyloctenium* lawns are common.

Potential errors

The previous chapters raised potential problems in extrapolating the results of the experiments. In the sand and solution culture experiments, it is not known whether the N supply at which growth differences between species were observed are equivalent to those which occur under natural conditions. Also, spatio-temporal variation in N supply which is likely to occur under natural conditions was not incorporated. In the field experiments, fertilization may not have increased the supply of N to the target species significantly, at least for *Digitaria* and *Urochloa*. Finally all experiments measured only short-term growth responses while longer term responses, incorporating reproduction and storage of assimilates, could not be measured. As a result, the importance of N supply may have been underestimated. However, none of these potential errors are considered large enough to discredit the results, and it is maintained that a high supply of N is not necessary for the occurrence of at least some lawn grass species.

Only four lawn grass species were used in the experiments but increasing the sample size would only strengthen this conclusion, as the variation in species responses reported could only increase by testing more species. For example, *Cynodon dactylon*, a species dominant on potential grazing lawns in other systems, showed a similar growth response to *Digitaria* in a different solution culture experiment conducted in the same growth chamber (Mafa 1999). From the data presented by Mafa, mean RGR was calculated on the assumption that the initial weights of the *C. dactylon* plants were the same as those of the *Digitaria* plants in the solution culture experiment (which is probably valid considering the similar morphology of the two species). The resulting calculation of maximum RGR was very similar to that of *Digitaria*, although a decrease in RGR (and an increase in RMF) occurred at a slightly lower N level than for *Digitaria*.

Potential limits to the supply and demand of N

In the introduction to chapter 2 a range of studies showing that heavy grazing leads to an increased supply of mineral N were cited. If N supply is universally high in heavily-grazed environments, this begs the question why lawn grasses such as *Digitaria*, *Sporobolus*, *C. dactylon* and possibly *Dactyloctenium* have not evolved adaptations to exploit a high supply of N. There are two potential explanations for this:

1. many lawn grasses are unable to exploit a high supply of N due to growth limitations imposed by other resources / stresses, or
2. an increase in mineral N supply is not a universal response to heavy grazing.

Exploring these points goes beyond the scope of this thesis. However, in reviewing the literature on grass-grazer interactions, some evidence was found regarding the latter, and as this contributes to the conclusion that high N supply is not a key process, it is discussed briefly.

The effect of grazing on N supply

Studies reporting an increase in the supply of mineral N in grass-grazer systems (cited in chapter 2) are limited to a few sites in the Serengeti and North America. However, a few studies from other sites do not support the pattern. Working in the cattle-grazed grasslands of the flooding pampa, Argentina, Lavado *et al.* (1996) found a reduction in the soil concentration of mineral N outside of grazing exclosures. In the Serengeti, the results of Belsky (1986a) show that although ammonium was significantly higher in lawn grass patches dominated by *Andropogon greenwayi* (relative to 18 year old exclosure), both ammonium and nitrate concentrations were significantly lower in neighbouring grazing lawn patches dominated by *Chloris pycnothrix*. There is therefore reason to believe that grazer-induced increases in mineral N supply are not universal but contingent upon specific environmental conditions. With reference to HUP, conditions which might restrict grazer-induced increases in rates of N mineralization include:

1. The dung deposition patterns of white rhino. This species creates dung middens, which could result in the export of N from lawns to the middens.
2. High rainfall. The potential for denitrification to outweigh grazer-induced increases in N mineralization in mesic environments was mentioned in chapter 3, while N losses in the form of nitrate leaching from urine patches, and denitrification in urine patches, and

ammonium losses from the grazers, are considered important in intensely grazed, mesic livestock systems (Jarvis 1996, 2000).

3. Tolerance of grazers to low-N forage (which is implied by the N responses of *Digitaria, Dactyloctenium,* and *Sporobolus*). If lawn grasses have lower leaf N concentrations than neighbouring bunch grasses, but still provide better forage as a result of higher leaf:stem and live:dead ratios, then the mechanism of increased mineralization through decreased litter C:N ratios (chapter 2) would not occur. Instead, grazing would lead to decreased rates of mineralization, as proposed by Ritchie *et al.* (1998).

Water

The single irrigation experiment conducted is not considered sufficient evidence to discard the hypothesis that a low supply of water is a key process limiting the distribution of grazing lawns. As mentioned in chapter 3, rainfall was above average when this experiment was conducted and the effect of a low supply of water was not actually tested. Considering the studies cited in the introductory chapter which indicate that grazing lawns are uncommon in the semi-arid grasslands and savannas of southern Africa, even where grazing intensity is high, the potentially key role of water deserves further attention. On the other hand, as extensive grazing lawns occur in the Serengeti in areas with a mean annual rainfall less than 500mm (McNaughton 1985a) – lower than many of the semi-arid parts of southern Africa (including the driest parts of HUP) - the importance of water cannot be taken for granted.

New ideas

Further key processes?

N toxicity

While any increase in N supply created by heavy grazing may not influence lawn grasses directly, it may affect them indirectly through toxic effects on bunch grasses. Thus N would be a key process which ameliorates competition from bunch grasses, in the same way that defoliation does. Tolerance of a high supply of N, rather than intolerance of a low supply, would then be an adaptation which lawn grasses share and which distinguishes them from the grazing-intolerant bunch grasses. This idea is supported by the vegetative growth responses of the four lawn grass species grown in solution culture, which indicated minimal stress at a very high N supply of 40mM (chapter 2 section 3). A high demand for N and high rates of N uptake by lawn grasses could then be a disadvantage, in that this could lower concentrations of N in the soil solution to levels acceptable to bunch grasses. While N toxicity would not be relevant in semi-arid systems, the absence of grazing lawns from mesic grass-grazer systems might be a result of abiotic conditions limiting the extent to which N supply is enhanced and / or improving the ability of bunch grasses to tolerate high concentrations of mineral N.

This idea of N toxicity to bunch grasses is in fact an old one – the retrogression of southern African highveld bunch grass communities observed to occur in response to fertilization was proposed to be a result of N toxicity as far back as the 1950's (O'Connor and Bredenkamp 1997). Some of these experiments reported that 'pioneer species' such as *Urochloa* and *Cynodon dactylon* increased with fertilization, quickly replacing 'climax' and 'sub-climax' bunch grass species (O'Connor 1985). The proposal by Tew *et al.* (1974) that NADP-ME C_4 species are sensitive to high levels of ammonium N was mentioned in chapter 2 (section 2), as was the sand culture experiment by Roux (1954) which showed reduced growth by two bunch grass species (*Trachypogon plurosus* and *Eragrostis curvula*) at higher levels of ammonium N supply. In a recent experiment involving relatively low levels of N, Snyman (2002) found that certain bunch grasses, including *Themeda triandra*, showed a similar, rapid disappearance.

Despite this evidence, N toxicity is unlikely to qualify as a key process. Firstly, toxicity only appears to occur for certain bunch grass species (usually of the tribe Andropogoneae) but not others (Snyman 2002). Secondly, whether grazing could increase mineral N levels to those created in the fertilization experiments cited above has yet to be established. Thirdly, there is evidence that two common lawn grass species *Digitaria argyrograpta* (O'Connor 1985) and *Cynodon dactylon* (Roux 1955) are as sensitive to a high supply of N as bunch grasses. Finally, it seems unlikely that the process would occur in the most mesic parts of HUP, where total N concentrations in the soil are high even below ungrazed bunch grass swards (chapter 3), and where no obvious effect of the N addition in the field fertilizer experiment (chapter 3) were observed. As with the N supply hypothesis, N toxicity may play a role in reducing bunch grass competition in mesic systems with, for example sandy soils (O'Connor 1985) or different bunch grass species, but is unlikely to be a key process controlling the distribution of grazing lawns in general.

Phosphorus

If N supply is not a key processes involved in the distribution of grazing lawns, it is difficult to speculate which other mineral resources might be. It has been shown to have a large effect on growth under controlled conditions for a range of species (Lambers *et al.* 1998), including two grazing lawn species from the drier parts of the Serengeti (McNaughton and Chapin 1985). In addition, fertilizer experiments have shown increased growth of C_4 bunch grasses (Kelk 1972; Rao *et al.* 1996), including *Panicum coloratum*, a species common on the drier grazing lawns of HUP (O'Connor 1985). However, the effects of P addition are usually far less than those of N in factorial fertilizer trials where both minerals are added (O'Connor 1985; Snyman 2002). Furthermore, as P availability is controlled to a certain degree by the same process as N availability (mineralization - Lambers *et al.* 1998), it is likely that N and P supplies would be closely correlated, even under conditions of heavy grazing. Hatch and Tainton (1990) found no differences in "plant available" P in a comparison of heavily-grazed and ungrazed patches in a mesic grassland in KwaZulu-Natal. Therefore, P supply is even less likely to be a key process than N supply.

Na toxicity

High levels of grazing have been associated with an accumulation of sodium (Na) in the soil (McNaughton *et al.* 1997a), and heavily grazed semi-arid systems may accumulate Na to the point that it becomes toxic to lawn grasses. This could explain the low cover of lawn grass species (and others) from the sodic sites of granitic landscapes in the lowveld of South Africa. However, *Sporobolus* occurs on brackish soils (van Oudtshoorn 1999) and even sodic sites (pers. obs.), and was observed to exude salt crystals on its leaves in the solution culture experiment - a feature of salt tolerant species (Lambers *et al.* 1998). Therefore, while 'Na toxicity' may exclude some species, it is unlikely to qualify as a key process restricting the distribution of all lawn grasses. As with N, it can be speculated that Na accumulation has the reverse effect, i.e. Na toxicity affects bunch grasses more severely, thus preventing competitive exclusion of lawn grasses. This might be a key process in mesic systems, but only if leaching was high enough to prevent any significant accumulation of Na.

Rooting space

While competition for space for nodal roots may be important for *Dactyloctenium* it cannot explain the exclusion of non-stoloniferous lawn grass species (such as *Sporobolus*).

Grazing-tolerance trade-offs

Searching for key processes using an alternative approach – identifying 'trade-off traits' associated with grazing tolerance – also produced no promising new key processes. Grasses and grazers have co-existed for approximately 45 million years (Stebbins 1981) and all show some degree of grazing tolerance, and posses a range of potential grazing tolerant traits (Kotanen and Bergelson 2000). However, a review of the literature on the effects of clipping on grasses revealed no traits unique to grazing lawn species, other than an ability to restrict defoliation losses by keeping a large proportion of leaf area close to the ground and out of reach of grazers (as suggested by McNaughton 1979). This is achieved by means of short stature (Coughenour *et al.* 1985b; Landsberg *et al.* 1999; Kotanen and Bergelson 2000), a small leaf area per tiller (Coughenour *et al.* 1985b), prostrate leaves (Coughenour *et al.* 1984a) and short leaves (McNaughton 1984; Landsberg *et al.* 1999). Besides the obvious trade-off associated with these morphological adaptations - an inability to compete for light with bunch grasses – there is no evidence of any additional 'trade-off traits'. Furthermore, in terms of other aspects of grass growth affected by clipping - root growth (Pretorius *et al.* 1974; Edroma 1985; Hodgkinson *et al.* 1989; McNaughton 1992; Chaieb *et al.* 1996; Dawson *et al.* 2000) and the remobilization of stored carbon and nitrogen (Hodgkinson *et al.* 1989; Danckwerts and Gordon 1990; Thornton *et al.* 2000) - no evidence was found showing that lawn grass species (or potential lawn grass species) possess any unique grazing-tolerant traits.

Many limiting processes but none key?

Ecology is a science filled with novelties and '… the production of counter-examples to generalities' (Shugart 1997, p20) and perhaps grazing lawn communities represent another example. The limited distribution of grazing lawns may be the result of each lawn grass species being excluded by a different resource / stress (or even a different set of resources / stresses) rather than a single 'key process' affecting all. Thus in the most mesic parts of HUP *Digitaria* may be excluded by competition for some soil resource other than N, and *Sporobolus* by another. *Urochloa* may be excluded by a low supply of N, *Dactyloctenium* by a shortage of space AG for nodal rooting imposed by the density of bunch grasses, and other lawn grass species such as *Chloris gayana* and *Eragrostis superba* excluded by yet more factors. This would mean that important ecological processes are orthogonal to frequent, non-selective grazing, i.e. the evolutionary radiation of lawn grasses divided the 'grazing tolerant' niche into a number of sub-niches, each filled by different species with unique resource requirements. This scenario is a far more complex one - a veritable 'reductionist's nightmare' – but there is some evidence to support it. A number of fertilizer experiments show that different (co-occurring) species respond to the addition of different minerals (Bobbink 1991; and others cited by Gleeson and Tilman 1992).

In a similar vein O'Connor's (1999) conclusion of a discussion on the management of rangelands is that important ecological processes are contextual, with different ones important in different cases. This may be the case regarding the distribution of grazing lawn communities, i.e. different environmental conditions may result in different ecological processes constraining the growth of lawn grasses. For example, while the supply of N in heavily grazed environments in the most mesic parts of HUP does not seem to be responsible for the absence of grazing lawns there, N may well be limiting in other systems with different soils, where other resources (such as P) are less available (such as on sandy soils).

A shortage of non-selective grazing?

To conclude this thesis, the most simple explanation for an absence of grazing lawns in the most mesic parts of HUP is explored, namely that the frequency of non-selective grazing is too low to ameliorate bunch grass competition. In other words, the model presented in chapter 1 may be valid in its most simple form, with no key processes other than non-selective grazing necessary for occurrence of a grazing lawn. This is implied by the results of the landscape experiment in chapter 4. In HUP, there are two potential scenarios which could produce a shortage of grazing in the most mesic parts of the park. Management interventions may have reduced grazer populations below carrying-capacity, and below the level needed for the formation of grazing lawns (i.e. the grass-grazer system in HUP is not at equilibrium). Alternatively factors other than a shortage of summer forage limit the growth of grazer populations (i.e. there is a shortage of grazing even when the system is at equilibrium).

HUP as a system in disequilibrium

While herbivores in the present area of HUP were probably afforded better protection in the 1800's than in most game reserves, animal numbers were nevertheless reduced in the previous century (Brookes and MacDonald 1983). The area did not escape the rinderpest epidemic of 1896, although its exact impact is unknown. Thereafter, the Hluhluwe section of the park was spared from a massive culling campaign designed to eradicate tsetse fly, but neighbouring areas were not. While white rhino most probably occurred in the Hluhluwe section historically, none were recorded there in the 1930's. Since then re-introductions have occurred, and the population continues to grow (game count data – Hluhluwe Research Centre). The other two common non-selective species, zebra and buffalo, also experienced population reductions. Buffalo were re-introduced in the 1930's and have increased since, but frequent removal and culling has prevented the population from reaching carrying capacity. The zebra population peaked in the 1960's and is therefore currently lower than it could be. Similarly, the population of specialist short-grass feeders (blue wildebeest, warthog and impala) peaked in the 1960's and have subsequently been subjected to regular culling. Together, these reductions suggest that grazer populations may well be below carrying capacity, at least in the Hluhluwe section of HUP where most of the mesic parts are found. Whether this is responsible for the absence of grazing lawns there is therefore a hypothesis worth testing. Finally, the slow net growth of the lawn grasses measured in the landscape experiment (chapter 4) show that even with frequent grazing, it could take many years for lawn grasses to recolonize areas previously ungrazed (or lightly grazed).

A shortage of grazing in a system at equilibrium

If the grass-grazer system within HUP is currently at equilibrium, what process prevents populations of non-selective grazers from growing to the point that they would produce grazing lawns? The distribution of drinking water can be ruled out, as there is an extensive network of rivers in the higher parts of HUP. Likewise, predation is unlikely to be important as white rhino are all but immune to this, while lion densities are low in the mesic parts of HUP. The more hilly topography of the mesic parts may play a role, as steep slopes are known to discourage grazers (Bailey *et al.* 1996), but white rhino, buffalo and zebra are frequently seen on even the highest slopes and ridges in the park. The remaining explanation is that population sizes are limited by supply of winter forage.

Domestic grazer population density has long been considered to be limited by dry season forage, at least in South Africa (Tainton 1988). Simulation models involving a range of grass communities, including highly productive ones representative of grazing lawns in HUP, suggest that this is the case for grazers in general (Owen-Smith 2002). If higher grazer densities are required to produce lawns in more mesic environments, but winter forage is no better than in the more arid parts where lawns do occur, the frequency of non-selective grazing would be prevented from rising above required level. While the quantity of winter forage is certainly no less than in the more arid parts, it may be the quality which is lacking. This could also explain the absence of lawns from other mesic grass-grazer systems in southern Africa, particularly the sourveld areas where winter forage is not adequate to support cattle. Relatively lower forage quality in the mesic parts of HUP, as compared to the drier parts where grazing lawns do occur, could be the result of bunch grasses allocating more biomass to structural materials in response to greater light competition (in turn, a result of faster growth caused by higher rainfall).

Low quality winter forage may not only reduce overall grazer populations, but also alter the composition of grazer communities. Buffalo can tolerate a low quality diet, and are the most abundant grazers in the mesic parts of HUP (game count data – Hluhluwe Research Centre). However, they do not generally graze bunch grasses as short as other grazer species (Perrin and Brereton-Stiles 1999), which probably limits the extent to which their grazing reduces light competition from bunch grasses.

Finally, even if grazer populations are higher in the more mesic parts of HUP, the spatio-temporal distribution of grazing (in the growing season) may differ from more arid parts. The higher frequency of fire in the more mesic parts (Balfour and Howison 2002) could be responsible for this, as burning causes grazers to concentrate on resprouting bunch areas during the first few months of the growing season in HUP (S. Archibald; unpublished data), as well as other grass-grazer systems (Vinton *et al.* 1993; Lutge 1995).

CONCLUSION

The broad aim of this thesis, to determine what limits the distribution of grazing lawn communities in HUP, was not achieved. However, results of the experimental work conducted do show that a high supply of N is not a key process necessary for the occurrence of lawn grasses. Water availability was not tested adequately and cannot be disregarded as a key limiting factor in semi-arid environments. In the mesic parts of HUP, lawn grasses suffer reduced growth as a result of competition from bunch grasses, but this is not severe enough to exclude them (in the short term, at least) when regular non-selective grazing occurs. Other growth factors, including N toxicity and other mineral resources, seem less likely than N supply to be key processes restricting the distribution of grazing lawns. It is speculated that the absence of grazing lawns is more likely the result of a multitude of ecological processes, with different resources / stresses important for different species or in different environments. Furthermore, in the most mesic parts of HUP and other mesic grass-grazer systems, the possibility that it is simply a shortage of non-selective grazing which excludes grazing lawns is also considered.

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