DISSERTATION

THE EFFECT OF INTRA-SEASONAL VARIATION IN PRECIPITATION ON THE PRODUCTIVITY OF GRASSES AND GRASSLANDS

Submitted by:

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WE HEREBY RECOMMEND THAT THE **DISSERTATION**
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ABSTRACT OF DISSERTATION

THE EFFECT OF INTRA-SEASONAL VARIATION IN PRECIPITATION ON THE PRODUCTIVITY OF GRASSES AND GRASSLANDS

Above-ground net primary productivity (ANPP), a fundamental process in terrestrial ecosystems, varies greatly between years particularly in grasslands. While annual precipitation is considered the primary determinant of ANPP in grasslands, much of the inter-annual variation in ANPP cannot be explained by total annual or seasonal precipitation alone. It was hypothesized that intra-seasonal variation in precipitation can account for some of the unexplained variation in ANPP, and that two other factors known to affect grassland ANPP (community composition and grazing) can alter the relationship between ANPP and precipitation. To test the importance of intra-seasonal variation in precipitation, long-term ANPP data for three C_4 grassland sites were analyzed. The effect of the average size, number and interval between rainfall events was determined and compared with that of annual precipitation. A field experiment was conducted at seven sites in undisturbed grasslands and savannas to investigate differences in growth responses to rainfall amongst co-occurring C_4 grass species, and to determine the effect of defoliation on these responses. A pot experiment was conducted to determine eco-physiological mechanisms responsible for differences in tolerance of water stress between two species dominant in mesic grasslands. It was found that intra-seasonal variation in precipitation can explain as much of the inter-annual variation in ANPP as annual precipitation, but that the relative importance of each varies between sites. Changes in grass community composition within sites had a relatively small effect on the relative importance of each. A lack of synchrony of intra-seasonal growth amongst co-occurring C_4 species was found in the field experiment. In the absence of grazing, intra-seasonal variation in growth rates was primarily determined by water availability at different depths for co-occurring species. Regular defoliation increased synchrony amongst species, and resulted in more similar growth responses to soil water. A number of above-ground traits were identified which could predict aspects of soil water utilization and defoliation tolerance. These results indicate that intra-seasonal variation in precipitation can be an important determinant of grassland ANPP, and that any effect of changes in community composition on ANPP-precipitation relationships is likely to be reduced by heavy grazing.

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CHAPTER 1

Introduction

Net primary productivity is a fundamental process of all ecosystems, being the process whereby energy enters a system and therefore ultimately the process that dictates the availability of energy for all other biota in the system. What determines net primary productivity has been a key question in ecology since the emergence of ecosystem ecology. The net primary productivity of grasslands has probably been more studied and is better understood than in other terrestrial ecosystems. In savannas with low tree cover, grass communities between tree and shrub canopies may function as patches of grassland, and studies of these communities contribute to the understanding grassland productivity (e.g. Pandey & Singh 1992, Augustine 2003).

While below-ground productivity is an important part of primary productivity, difficulties in its measurement have not enabled the advances made in studies of above-ground productivity. The above-ground net primary productivity (ANPP) of grassland ecosystems is usually simply measured as the total above-ground biomass at end of the growing season. Further bias towards measuring ANPP may stem from the fact that it is above-ground biomass that provides forage for grazing ungulates and fuel for fires. Both these "consumers" can have important effects on ecosystem structure and function (Harrington & Pratchett 1974, McNaughton 1985, McNaughton *et al.* 1993, Milchunas & Lauenroth 1993, Collins & Steinauer 1998, deBruyn & Scogings 1998, Knapp *et al.* 1998, Snyman 2003) making them relevant to the management of both natural grasslands and rangelands (i.e. grasslands grazed by domestic livestock).

From all the various studies of the ANPP of grasslands and savannas, annual precipitation has emerged as the most important factor controlling grassland ANPP. Despite the apparent ubiquity of the primary effect of precipitation, studies that relate ANPP to annual precipitation do not provide adequate answers regarding determinants of ANPP, particularly in the face of global climate change. These inadequacies are evident from low r^2 values for linear regressions of ANPP against precipitation. Notwithstanding potential errors of underestimation that may occur when using peak standing biomass as a measure of ANPP (resulting from the loss of senesced material during the growing season - Scurlock *et al.* 2002) there a number of potential problems with our current knowledge on the effects of precipitation on ANPP:

1. In "spatial studies" where time averaged ANPP and precipitation are compared for a range of sites (e.g. Le Houerou *et al.* 1988, Risser 1988, McNaughton *et al.* 1993, Ni 2004) differences in site-specific factors - such as soil type or grass species composition - are usually confounded with precipitation and may obscure its effect.
2. "Temporal studies" involving regressions of a series of annual ANPP and annual precipitation data for a particular site provide a more direct test of the effect of precipitation (e.g. Lauenroth & Sala 1992, Nippert *et al.* 2006), but regressions are often weak and sometimes non-linear.
3. Factors that may interact with precipitation to affect ANPP are not usually taken into account. Most studies involve sites with constant species composition, and either no grazing and no fire, or some fixed level of these. Notable exceptions are the work of McNaughton (1985) who showed that intense grazing modified

ANPP-precipitation relationships, Knapp *et al.* (1998) who show that regular burning reduces ANPP in dry years, and O'Connor *et al.* (2001) who found different slopes of ANPP versus annual precipitation for different plant communities at the same site.

4. Precipitation is quantified simply as an annual or seasonal total. However, precipitation is never constant through the year (particularly in grassland and savanna regions) and much of the explanatory power of precipitation may be lost by not considering the effects of the temporal variation of precipitation within the growing season. Some recent experimental work indicates that the timing of precipitation can affect grassland ANPP (Knapp *et al.* 2002).

Objectives of the dissertation

The overall aim of this dissertation was to better understand the effect of precipitation on ANPP, by investigating two potentially important factors controlling ANPP that have received little attention in the past. These are intra-seasonal variation in precipitation, and the effect of the composition of primary producers, i.e. grass community composition, on the relationship between precipitation and ANPP. The effect of herbivory, which is known to affect ANPP in many ecosystems, was also included in the form of investigating the potential interaction effect of grazing and community composition on ANPP-precipitation relationships.

Precipitation distribution

In most grassland ecosystems, annual precipitation occurs primarily as rainfall during the growing season. Mediterranean grasslands are an exception, and these are not dealt with in this dissertation. Instead the focus is on ecosystems where the majority of rainfall occurs during the growing season. Rainfall during the growing season is often erratic – due to its origin in convective storms – with large variation in the size of rainfall events and in the intervals between rainfall events. This creates a temporally variable supply of water for primary producers, and grasses are clearly affected by this, often showing periods of rapid growth followed by periods of water stress and little or no growth, within the growing season. Furthermore, the temporal patterns of rainfall can lead to spatial variation in soil water supply, with different rainfall sizes and intervals affecting the degree of infiltration down the soil profile.

The potential effect of intra-seasonal variation in rainfall on ANPP has been described for arid and semi-arid systems (Noy-Meir 1973). However there have been only a few attempts to quantify intra-seasonal variation in rainfall and test for its effect on ANPP empirically (Knapp *et al.* 2002, Lauenroth & Sala 1992, Wiegand *et al.* 2004) as has been done for precipitation totals. This may be because intra-seasonal variation in precipitation is difficult to quantify. While annual or seasonal precipitation is quantified as a simple total, intra-seasonal variation in rainfall incorporates many variables (rainfall size, rainfall spacing, cumulative rainfall size) which are all potentially correlated to each other and to total precipitation.

Community composition

Many applied studies, investigating forage supply on rangelands, have established that grass species composition can have a substantial effect on ANPP, as have a few studies of natural grasslands (e.g. Kahmen *et al.* 2005). However, few have shown that differences in species composition can alter the effect of annual precipitation on ANPP, O'Connor *et al.* (2001) being an exception. Grass species have presumably evolved adaptations to cope with spatio-temporal variation in soil water availability (Williams *et al.* 1998), and if these adaptations differ amongst co-occurring species, then large changes in species composition should cause changes in the effect of both total precipitation and intra-seasonal variation of rainfall on ANPP. The replacement of perennial grasses by annual grasses, following heavy grazing and / or the introduction of exotic forage species has been shown to alter soil water utilization and presumably precipitation relationships (e.g. Holmes & Rice 1996). The focus for this dissertation is on perennial grasslands which constitute the majority of grasslands with economic and conservation value.

Grazing

Grazing has been an integral part of grassland ecosystems since the evolution of grass plants (Stebbins 1981), and occurs in the vast majority of grasslands and savannas, in the form of native or domestic ungulates (McNaughton *et al.* 1991). A complete understanding of ANPP-precipitation relationships in grasslands should therefore consider any additional effects created by grazers. Furthermore, manipulating ungulate grazer densities is one of only few means to manage grasslands, both in conserved and agricultural environments, but this requires a thorough understanding of grazer effects.

While understanding the effects of grazing on ANPP *per se* was not an aim of this dissertation, the potential effect of non-selective grazing modifying ANPP-precipitation relationships was investigated.

Key questions

The following questions were addressed using existing long term data, a field experiment and a pot experiment.

1) **Does intra-seasonal variation in rainfall affect ANPP?**

   To separate the effect of total precipitation from intra-seasonal variation in rainfall requires either a rainfall manipulation experiment (e.g. Fay *et al.* 2000) or the use of long-term data that has sufficient variation in rainfall patterns to disentangle the effects of intra-seasonal variation from total amount. The former approach was not considered viable for a PhD research project, due to the costs involved and the duration that such an experiment might require to produce meaningful results. Instead long-term data were obtained for three grassland sites, and the effects of both precipitation total and intra-seasonal variation analyzed (**chapter 2**).

2) **Does community composition alter relationships between ANPP and precipitation?**

   The long-term data above included data for communities differing substantially in species composition, allowing an initial investigation of the role of species composition in determining ANPP responses to precipitation totals and intra-season variation. These results are also presented in **chapter 2**.

The role of species composition was investigated in more detail using a field experiment, described in **chapters 3** and **4.** The conceptual model underlying the design of the field experiment was that the ANPP of a given community of primary producers is simply the sum of the productivity of the individuals making up the community. The above-ground production of individual grasses growing under natural conditions was therefore regularly measured, and intra-seasonal growth patterns used to determine whether co-occurring species differed in terms of their growth responses to intra-seasonal variation in rainfall.

3) Does grazing alter the effects the community composition on ANPP-precipitation relationships?

The potential effect of grazing was investigated by incorporating a clipping treatment in the field experiment described in **chapters 3** and **4**. By repeatedly clipping certain individuals, differences in the effect of regular defoliation between co-occurring species determined.

4) Are there functional groups that show similar above-ground growth responses to precipitation and defoliation?

If co-occurring species do respond differently to precipitation, there may be a continuum of responses or species may cluster in groups with similar responses. As physical limitations and phylogenetic constraints limit the range of adaptations that may evolve for various plant functions (such as water uptake and tolerance of water stress) it has been argued that species do cluster into groups with similar function (i.e. functional groups - Gitay & Noble 1997, Lavorel *et al.* 2005). Trees and grasses are generally treated as functional groups due to differences in resource acquisition and responses to fire and herbivory (Sankaran *et al.* 2004), and C_3 and C_4 grasses are usually treated as separate functional groups, in terms of their responses to water, nitrogen, burning and temperature (Knapp & Medina 1999, O'Connor & Bredenkamp 1997). In **chapter 5** data collected in the field experiment above were used to categorize perennial C_4 grasses into functional groups relating to soil water utilization and tolerance of defoliation.

5) What functional traits define such functional groups?

During the course of the above-field experiment, measurements were made of easily-measured traits that might explain why different species respond differently to intra-seasonal variation in rainfall and grazing. The value of these traits, in terms of classifying species according to the established functional groups, and in terms of the actual functions of the traits, is presented in **chapter 5**.

Finally, a pot experiment was conducted to further investigate plant traits that allow grasses to cope with intra-seasonal variation in water availability. Individuals of the two dominant species of the mesic grasslands of North America were exposed to severe water stress, and traits that affected their tolerance of water stress were identified. This experiment is described in the **chapter 5.**

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CHAPTER 2

Intra-seasonal precipitation patterns and aboveground productivity in three perennial grasslands

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SUMMARY

1. Relationships between above-ground net primary productivity (ANPP) of grasslands and annual precipitation are often weak at the site level, with much of the inter-annual variation in ANPP left unexplained. A potential reason for this is that the distribution of precipitation within a growing season affects productivity in addition to the total amount.

2. We analysed long-term ANPP data for three southern African temperate grasslands (mean annual precipitation ranging from 538 – 798 mm) to determine the effects of precipitation event size, number and spacing relative to seasonal totals.

3. Ungrazed, non-manipulated treatments at each site showed contrasting results despite sharing a common, dominant species. At the driest site, a model combining average event size and number of events per growing season provided a substantially better fit to the ANPP data than precipitation amount (seasonal total). At the wettest site, the interval between events was the most important precipitation variable. Precipitation distribution was not important at the intermediate site where amount was the best predictor of ANPP. A limit to the size of precipitation events efficiently utilized for ANPP was evident for the driest site only.

4. At each site, experimental treatments that altered species composition and soil fertility had little effect on ANPP-precipitation relationships. The lack of consistency in the relative importance of the precipitation variables among sites suggests that local, edaphic factors modify ANPP-precipitation relationships.

5. This analysis demonstrates that the distribution and size of precipitation events can affect ANPP independent of precipitation amount. As altered precipitation regimes are

forecast by global climate models, the sensitivity of ecosystems to precipitation distribution should be considered when predicting responses to climate change.

6. While mean values of precipitation, and other ecosystem drivers, are typically used to predict function at the level of whole ecosystems, our results show that more complex measures of environmental variability may be required to understand ecosystem function, and to increase the accuracy of predictions of ecosystem responses to global change.

INTRODUCTION

Determining the controls on net primary productivity, a fundamental process in all ecosystems, is one of the founding goals of ecosystem ecology. The above-ground net primary productivity (ANPP) of grasslands has been relatively well studied, with inter-annual variation in ANPP widely regarded to be primarily determined by inter-annual variation in precipitation quantity. Regional-scale meta-analyses, relating mean peak biomass of sites to mean annual precipitation, have confirmed this relationship for most of the grassland and savanna regions of the world (Deshmukh 1984, Epstein *et al.* 1997, Le Houérou, Bingham & Skerbek 1988, Risser 1988, Sala *et al.* 1988, McNaughton, Sala & Oesterheld 1993, Ojima *et al.* 1999, Ni 2004). While these analyses may be useful for predicting regional scale effects of projected global change (e.g. Burke *et al.* 1991), site-specific analyses are required to predict the effects of global change at the local scale and to gain a mechanistic understanding of how precipitation affects ecosystem function. Indeed, long-term experiments at the site level have generated sufficient data to allow for more mechanistic assessments of the effect of annual precipitation on ANPP for perennial grasslands around the world (Smoliak 1986, Le Houérou *et al.* 1988, Lauenroth & Sala 1992, Briggs & Knapp 1995, Knapp *et al.* 1998, Jobbagy & Sala 2000, O'Connor *et al.* 2001, Chidumayo 2003, Knapp *et al.* 2006, Nippert *et al.* 2006). However, while most precipitation-ANPP relationships are significant at the site level, much of the variation in ANPP is not accounted for, even in long-term studies, and relationships often appear non-linear.

A potential reason for poor relationships between precipitation and ANPP is that the distribution of precipitation within a growing season affects ANPP independent of the total amount. It has long been suggested that the majority of plant productivity occurs in the form of short-duration pulses following rainfall events, at least for semi-arid and arid systems (Noy-Meir 1973). There is likely to be an optimal distribution of such pulses for ANPP, i.e. an optimal distribution of precipitation event sizes and of the intervals between them. For example, Lauenroth & Sala (1992) found that precipitation events of 15mm to 30mm were responsible for most of the effect of precipitation on ANPP for a short grass steppe site (although the potential importance of small events <5mm has also been emphasised - Sala & Lauenroth 1982). For mesic tallgrass prairie, Knapp *et al.* (2002) found that extending the intervals between precipitation events - without changing the total precipitation for the season - reduced ANPP by about 10% over four years in a rainfall manipulation experiment. The optimal distribution of events sizes and intervals is likely to vary from one ecosystem to the next, or even between different communities in the same system, depending on rates of run-off, infiltration, evapotranspiration, and the ability of dominant species to tolerate water stress between events. Global climate change models predict not only changes in annual precipitation (Houghton *et al.* 2001) but also increases in “extreme events”, with precipitation more likely to fall in larger but less frequent events (Easterling *et al.* 2000, Gordon *et al.* 1992, Meehl *et al.* 2005). Analyses of long-term climate data from regions throughout the world indicate that such increases in extreme precipitation regimes have already begun (Karl *et al.* 1995, Groisman *et al.* 1999, Plummer *et al.* 1999, Easterling *et al.* 2000, Domonkos 2003). These changes warrant more detailed investigation of the effects of precipitation distribution patterns on productivity.

To determine whether the distribution of precipitation does affect ANPP independent of precipitation amount, we analysed long-term ANPP data for three grasslands dominated by C_4 perennials, each differing in mean annual precipitation and soil type. At all three sites low soil water, not low temperature, determines the dormant season (empty bucket systems *sensu* Knapp *et al.* 2006). Standing biomass was removed every year at each site, eliminating any potential effects of carried-over biomass (Knapp & Seastedt 1986, O'Connor *et al.* 2001, Haddad *et al.* 2002, Chidumayo 2003) which allowed for a focused analysis of the effects of current season precipitation. In addition, treatments originally implemented at each site to address rangeland management questions have created communities differing remarkably in species composition and soil fertility. These long-term data were used to address three questions: 1) does the distribution of precipitation affect ANPP independent of amount, 2) does this effect differ between different grassland ecosystems, and 3) does this effect differ within an ecosystem, due to differences in species composition and soil fertility?

METHODS

Site and data descriptions

Long-term data for ANPP and precipitation were obtained from three sites in South Africa, referred to as Bloemfontein, Towoomba and Ukulinga. Data for Bloemfontein are from a long-term experiment established in 1977 at an agricultural research farm (Sydenham) near Bloemfontein, and in 1995 at the campus of the University of the Free State, 5km away (26°15`E 28°50`S, altitude 1350m). The same experimental design was used at both sites, and both had similar soils (a sandy clay loam) and the same C_4 grass community (soils and species composition for each site are described in more detail by O'Connor *et al.* 2001 and Snyman 2000). Data from both locations were treated as a single set. Mean monthly temperatures on the campus ranged from 17°C in July to 33°C in January. Three treatments were established in 1977 with the goal of maintaining plant communities representing vegetation in good, moderate and poor states, as would be created by grazing regimes of increasing intensity. The medium and poor condition communities were created and maintained by removing certain species by hand. The existing (ungrazed) vegetation at the start of the experiment was considered in good condition and left unchanged. Few individuals had to be removed after the first few years. There were three replicates of each treatment, each consisting of a 2m x 15m plot (treatments were randomly assigned to plots). ANPP was estimated as peak standing biomass, measured by clipping the aboveground biomass of eight 0.25m^2 quadrats, placed randomly in each plot, to a height of 3cm at the end of each growing season.

Entire plots were subsequently cut to the same height in July each year, before the start of the next growing season.

Results are presented for the good condition and poor condition plots only, as those for the moderate condition plots were similar to those for the good condition plots. The good condition plots (referred to hereafter as the control plots) were dominated by *Themeda triandra* Forssk., with *Eragrostis chloromelas* Steud. and *Elionurus muticus* (Spreng.) Kuntze. subdominant, and the poor condition plots by *Tragus koelerioides* Asch., with *Aristida congesta* Roem. & Schult. subdominant. Data were obtained for 29 growing seasons (1977 to 2006). Daily precipitation data were obtained from the Bloemfontein airport and the campus of the University of the Free State.

Data for Towoomba are from a fertilisation trial conducted from 1949 to 1990 at the Towoomba Research Station (28°21′E 24°54′S, altitude 1134m) near Bela-Bela. Soils are a red, sandy loam classified as Hutton form (Donaldson *et al.* 1984). Summers are hot with a mean monthly maximum of 30.3°C in December, and winters cool (July mean monthly minimum of 3°C with frequent light frosts. The trial consisted of a 5 x 3 factorial design of nitrogen (N) and phosphorus (P) addition with four replicates each randomly assigned to a block. The site was cleared of all woody species at the start of the experiment. Each replicate consisted of a 6.4m x 9.1m plot, mown to a height of approximately 5cm annually when the majority of grasses were in the early flowering stage. Mowed material was air dried and weighed. 13 years into the experiment large changes in species composition, the concentrations of various soil nutrients, and soil pH, compaction and infiltration rates were recorded in response to all the fertilisation treatments (Donaldson *et al.* 1984). Analyses for the Towoomba site were restricted to data from the control plots, and the fertilisation treatment that produced the greatest increase in productivity, referred to as the P2N3 treatment. This treatment received an annual total of 151.4 kg N ha^{-1} as NH_4SO_4 applied three times during the growing season, and 37.9 kg P ha^{-1} as super phosphate applied annually in November. In the control plots *Themeda triandra*, *Cymbopogon pospischilii* (K. Schum.) C. E. Hubb. and *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult. remained dominant, while in P2N3 plots species of the tribe *Andropogoneae* disappeared and were replaced primarily by *Panicum maximum* Jacq. Due to missing data, only data for 27 growing seasons were used (1949-1957, 1960-1975, 1977-1981). Daily precipitation data were collected on site.

Data for the Ukulinga site came from a long-term fertilisation experiment located at the Ukulinga Research Farm (29°24'E 30°24'S, altitude 845m) of the University of KwaZulu-Natal, Pietermaritzburg. The experiment is situated on top of a plateau with a lithology of Ecca group shales of the Karoo sedimentary sequence. Soil depth is approximately 60cm. Soils are acidic and relatively infertile, and are classified as Westleigh form (plinthic paleustalf; Fynn & O'Connor 2005). Summers are warm to hot with a mean monthly maximum of 26.4°C in February, and winters are mild with occasional frost and a mean monthly minimum of 8.8°C in July. The experiment was established in 1951 to determine the effect of fertiliser (various combinations of N, P and lime) on the productivity and composition of natural grassland. Major changes in species composition were observed within a few years of commencement of the treatments, and the various treatments now represent ecosystems differing markedly in nutrient availability, productivity and species composition (Fynn & O'Connor 2005). There were three replicate 2.7m x 9.1m plots per treatment. ANPP was measured twice each year, in December (the middle of the growing season) and in February (towards the end of the season), by mowing a 2.1 m strip in each plot. Mowed material was weighed fresh, and this weight converted to dry mass using a subsample dried to constant mass.

Analyses were restricted to data from the control and the treatment receiving the most N and P, referred to as the N3P treatment, which had the highest mean ANPP of all the treatments over the study period. This treatment received an annual total of 211.7 kg N ha^{-1} as NH_4NO_3 applied bi-annually and 28 kg P ha^{-1} as phosphate applied annually. The control plots were dominated by *Themeda triandra* and *Tristachya leucothrix* Nees, and the N3P plots by *Panicum maximum* with *Eragrostis curvula* Schrad. (Nees) subdominant (Le Roux & Mentis 1986, Fynn & O'Connor 2005). Daily precipitation data was collected on site. Due to missing data, information from only 30 growing seasons could be used (1950-1963, 1965-1979, 1997/8 and 1999/2000).

Precipitation variables

The effect of precipitation amount was evaluated using growing season totals. The effect of precipitation distribution was evaluated using three variables: average precipitation event size, number of precipitation events and the average interval between precipitation events over a growing season. Average event size and event number together determine precipitation amount. However these variables may affect ANPP independent of amount, and of each other, depending on how efficiently primary producers utilise events of various sizes, and how vulnerable they are to infrequent rainfall events. Event number is related to average interval as fewer events will always result in longer intervals. However, average interval provides a more direct measure of the (temporal) spacing of precipitation events and was included in analyses for a better estimate of this effect. For analyses where event size appeared to be important, an additional variable was calculated by "capping" events to 25mm (i.e. setting all events >25mm to 25mm and recalculating seasonal totals). 50mm capped totals were calculated in a similar way. This was done to determine if there was a saturating effect of event size (that would occur if substantial run-off or infiltration below the rooting zone followed large events).

For Bloemfontein the growing season was arbitrarily defined as the 1st September to 30th April, for Towoomba as the 1st September to the end of the month of cutting, and for Ukulinga from the 1st September to the date of the 2nd cut. All the precipitation distribution variables were calculated for the duration of these growing seasons. A precipitation event was defined as all precipitation $\geq$1mm falling on consecutive days. Precipitation intervals were calculated as the number of days between events.
The distributions of events and intervals were strongly skewed to the right for each growing season (and when combined for all seasons – Fig 2.1). Growing season averages were therefore calculated as geometric means.

Statistical analyses

Simple linear regressions were used to test the effect of seasonal total for each treatment at each site. As seasonal total was often correlated with precipitation distribution variables, the independent effects of the various variables were not tested together in a single model. Instead separate models were used to evaluate the effects of either seasonal total or precipitation distribution. Models for each treatment at each site were then compared on the basis of linearity, outliers and the corrected Akaike's Information Criterion (AICc). Outliers were judged using the standardised DFFITS statistic. DFFITS is calculated by refitting models after removing a particular datum, and indicates how much the partial regression coefficients in a model change (in standardised units) when an outlier is excluded. AICc estimates the likelihood that a model is correct, given the data, relative to alternative models. The AICc for each model was calculated from the mean-square-error of a least squares regression, and these values used to calculate the relative likelihood of the particular models under comparison, following Burnham & Anderson (2002). The relative likelihood is the probability that a particular model, in a set of compared models, provides the best fit to the data. Two multiple regression models were used to test for the effect of precipitation distribution: one combining average event size and event number, and one combining average event size and average interval. Event number and average interval were not tested together as these variables were always correlated. Average event size and event number, and average event size and average interval, were not correlated in any of the analyses, and variance inflation factors were never greater than 1.1. The inclusion of an interaction term did not improve the fit of any of these models. All analyses were performed using Statistica v6.1 (Statsoft Inc., Tulsa, USA).

RESULTS

Mean annual precipitation across the three sites varied from amounts typical of semi-arid to mesic grasslands (Table 1). The ranking of the sites according to precipitation amount reflected differences in precipitation distribution: Ukulinga, with the highest precipitation, had the most precipitation events but the smallest average event sizes and the shortest intervals between events (Fig. 2.2). Bloemfontein, the driest site, was the opposite and Towoomba was intermediate. At each site, inter-annual variation in ANPP was large and the CV of ANPP always greater than that of the total annual precipitation (Table 2.1). Bloemfontein had the greatest inter-annual variation in precipitation, although the CV of ANPP was no greater than that at Towoomba, where the CV of precipitation was lower. The poor condition plots at Bloemfontein had greatly reduced ANPP and rain use efficiency (RUE, *sensu* Le Houérou 1984), and showed greater inter-annual variation in these. Ukulinga had the greatest ANPP, the least inter-annual variation in ANPP and the greatest RUE. At both Towoomba and Ukulinga fertilisation with N and P increased productivity, by almost 280% above that of the control plots at Towoomba and 75% at Ukulinga. Fertilisation also increased inter-annual variability in ANPP, particularly at Ukulinga.

Productivity of the control plots at each site showed different responses to precipitation distribution, with precipitation distribution variables providing a better fit to the ANPP data than precipitation amount at Bloemfontein and Ukulinga, but not at Towoomba (Fig. 2.3). At Bloemfontein, the driest site, incorporating the effects of event size and event number provided the best model (Table 2.2). While precipitation amount showed a linear effect on ANPP, a large outlier resulted in a relatively poor fit. The multiple regression combining event size and number could account for the low ANPP in the outlier year, and explained more of the variation in ANPP. Standardised parameter estimates were similar for each variable, and maximum productivity was only obtained in years with both large events and many events. Event size and interval produced a weaker model revealing little effect of the spacing of precipitation events. Capping precipitation to 25cm produced a model similar to event size plus event number, and better than precipitation amount (r^2 improved from 0.46 to 0.66 and no clear outliers). At Towoomba, the site with intermediate precipitation, precipitation amount had a linear effect on ANPP with no outliers (Table 2.2). Neither of the precipitation distribution models provided a substantial improvement to this model, although event size alone gave a similar fit with an almost identical AICc. Capping event sizes to 25mm or 50mm did not improve on the amount model. The effect of precipitation amount at Ukulinga, the wettest site, was weak (Table 2.2). Event size plus event number improved on the amount model, again accounting for a large outlier. The event size plus interval model showed an even greater improvement and clearly provided the best fit. Standardised parameter estimates indicated a greater effect of interval than event size.

Differences in productivity-precipitation relationships between the various treatments at each site were comparatively small. For the poor condition plots at Bloemfontein, event size plus event number again provided the best fit, but less of the inter-annual variation in the ANPP could be explained. Precipitation amount again contained an outlier and appeared to be non-linear, with ANPP often well above average in years with average rainfall. Capping events to 25mm improved on the amount model only slightly, while capping to 50mm produced a substantial improvement (r^2 increased from 0.32 to 0.42 and the relationship was more linear). At Towoomba, ANPP for the fertilised plots was similar to that for the control plots in dry years, but consistently greater in wet years (resulting in the substantially greater mean and variance). Precipitation amount had a strong linear effect on ANPP, with an r^2 value more than twice that for the control plots (Table 2.2). Capping event sizes to 25mm or 50mm did not improve on this model, and event size alone did not produce as good a fit. The greater and more variable ANPP of the fertilised plots at Ukulinga also resulted from greater maximum values, relative to the control plots. However, greater productivity did not necessarily occur in the wettest years, and precipitation amount provided a very poor fit to the ANPP data (Table 2.2). The precipitation distribution models provided large improvements, although r^2 was still low. A model with interval alone provided as good a fit as the combination of event size and interval.

DISCUSSION

Patterns of precipitation events within a growing season are complex and the variables used to quantify them are likely to be correlated with the amount of precipitation, as was the case in this study. This precludes a simple test of the relative importance of the amount and distribution, such as estimating their effects in a multiple regression model. However, the comparison of alternate models using a suitable criterion such as AIC – an approach now gaining popularity in ecology (Johnson & Omland 2004, Richards 2005) – revealed that variation in precipitation event sizes, numbers and intervals can explain inter-annual variation in ANPP better than seasonal precipitation totals.

The control plots at each site in this study differed substantially in their precipitation-ANPP relationships, with event sizes important at the driest site, the spacing of events important at the wettest site and neither important at the intermediate site. These differences occurred despite a common dominant species (*T. triandra*) at all three sites. Likewise results for the fertilised plots at Towoomba and Ukulinga differed despite both being dominated by *P. maximum*. Furthermore, large changes in species composition created by the various treatments had little effect on model selection within sites. The importance of precipitation distribution relative to amount, and of the precipitation variables relative to each other, therefore appears to be more a function of edaphic factors - such as soil texture, depth or fertility - than species composition. These results also indicate that certain species are able to dominate grass communities in environments with very different rainfall regimes.

At Towoomba the efficiency with which primary producers utilised precipitation was affected little by the distribution of the precipitation. The relatively sandy soil at this site has a high infiltration rate (194 mm/hour for the control plots - Donaldson *et al.* 1984) and run-off is likely to be minimal, even following large precipitation events, allowing primary producers access to most of the precipitation that falls. The strong relationship between event size and ANPP observed for the control plots, as well as the lack of an effect of capping large events, suggests that larger events are actually required for maximum productivity. Infiltration to deeper soil layers may therefore be necessary for the dominant species to obtain maximum productivity. The lack of effect of event number and average interval revealed that the spacing of events is not important under the current climate regime. Either rates of evapotranspiration are low enough to prevent significant drying of the soil between events, or the dominant species tolerate water stress well and maintain most of their biomass when soils dry between events. Low rates of evapotranspiration seem unlikely as the site experiences hot temperatures during the summer. Furthermore, the lack of effect of precipitation distribution was most evident for the fertilized plots where productivity is greater and rates of transpiration presumably higher. The particularly strong relationship between ANPP and precipitation amount for the fertilized plots was the result of far greater productivity in wet years. This may have been a consequence of the altered species composition as *P. maximum* (the dominant species) can grow very rapidly when nutrients and water are readily available (Pretorius, Grunow & Rabie 1974, Healey *et al.* 1998).

At Bloemfontein and Ukulinga precipitation distribution variables provided a better fit to the ANPP data than precipitation amount, but for different reasons. At Bloemfontein, both larger events and many events were required for maximum productivity. This may indicate that a threshold of soil water at a certain depth is required for optimal growth (with the threshold only reached after a large number of small events, or a few large events). Wilting during the growing season occurs regularly at this site (Snyman 1998) but as the average interval between events did not affect ANPP, the dominant species are presumably tolerant of this. Tolerance of water stress by the dominant primary producers therefore appears to be an important factor at both Bloemfontein and Towoomba, buffering productivity against the negative effects of long precipitation intervals.

In contrast to Towoomba, an upper limit to the size of events that contributed effectively to ANPP was evident at Bloemfontein. The main reason for the relatively weak effect of precipitation amount, for the control plots, was an outlier season that contained a few large events resulting in a large seasonal total but only moderate ANPP. As capping precipitation to 25mm greatly improved the relationship between amount and ANPP, the limit to the size of events that can be effectively utilised actually appears to be much lower than many of the events recorded. The effect of event size on ANPP at Bloemfontein therefore appears to be two-fold: larger event sizes up to about 25mm increase growth and therefore RUE, but event sizes above about 25mm are less effective and reduce RUE. The lower RUE for the poor condition plots reflects the inability of the dominant species to effectively utilize precipitation in most years (Snyman 1998, 1999). This also explains the weaker relationship between precipitation and ANPP. The lower

RUE was mainly a result of lower basal cover, not species composition, as differences in ANPP per unit basal cover between the good and poor condition plots are much less than the differences in ANPP alone (Wiegand *et al.* 2004).

The potential importance of the spacing of precipitation events was revealed for Ukulinga, the site with the highest precipitation and the shortest intervals between precipitation events. The low proportion of inter-annual variation in ANPP that could be explained for the Ukulinga plots is probably a result of the high frequency of events preventing water shortages in most growing seasons. This would result in high productivity in most years and can explain the low inter-annual variation in ANPP (for the control plots) and the weak relationships between precipitation amount and ANPP. Only when an unusually long interval between events occurs would water stress develop and productivity slow (thus the relatively strong effect of average interval on ANPP). For the control plots, the effect of event size suggests that larger events are required to eliminate water stress following a longer interval. These results indicate that the dominant species in the control plots at Ukulinga do not tolerate water stress well – the opposite conclusion to that reached for Bloemfontein and Towoomba, despite all three sites sharing a common dominant species. A potential explanation for this is that water stress is more severe at Ukulinga, when it does occur. The soil at Ukulinga is relatively shallow and this may limit storage of precipitation between events. Furthermore, greater clay content may result in lower soil water potentials even with moderate drying. Rates of evapotranspiration may also be higher at Ukulinga, given that productivity is higher.

The results for the control plots at Ukulinga are consistent with those found at the Konza Prairie Biology Station (USA), another mesic grassland on a finely textured soil, where longer intervals between events reduced ANPP (Knapp *et al.* 2002). Studies in other mesic grasslands are needed to confirm if the effect of the spacing of precipitation is a general pattern for mesic grasslands, or only occurs at sites with fine textured soils.

The contrasting best-fit models found for the three sites (Fig. 3) indicate that there may not be a common pattern of ANPP responses, for C_4 grasslands in general, to predicted changes in precipitation regimes. Rather, responses may be contingent on local edaphic factors. At Towoomba the apparent lack of effect of large events and long intervals suggest that productivity will be little affected by larger but less frequent precipitation events. An obvious caveat is that climate change may bring larger events and fewer events than were ever experienced in the duration of this study (Nippert *et al.* 2006). While there must be an upper limit to the size of events that can be effectively utilized, it was not evident in these data. The lack of an effect of capping events indicates that even events >50mm make a significant contribution to ANPP. Furthermore, the growing season with largest average event size also had the largest control plot ANPP. At Bloemfontein, increases in the frequency of large events, or larger individual events, can be expected to reduce RUE. The results for the poor condition plots suggest that heavy grazing will not change this response qualitatively. For Ukulinga, reductions in RUE are also likely, as a smaller number of precipitation events will result in longer intervals and a greater frequency of periods of water stress.

Huxman *et al.* (2004) found that RUE converges to a similar minimum during dry years, for a wide range of ecosystems representing most terrestrial biomes. Our results contrast with Huxman *et al.*, in that there was not a decrease in average RUE with mean annual precipitation across the three sites studied (Table 1), nor convergence to a similar RUE in dry years for all the plots included in our analyses (data not shown). Broad patterns that emerge from comparisons across large gradients in productivity and mean annual precipitation may overshadow site-level effects of precipitation distribution or site-specific factors on RUE. However, our results do show that these effects can be large enough to create substantial differences in RUE between ecosystems within the same biome and, for prediction at the site level, may be more important than broad scale comparisons.

Conclusions

Annual or seasonal totals of precipitation provide a simple and convenient means to evaluate the effect of precipitation on ANPP, but the more complex effects of precipitation distribution should not be overlooked, particularly considering the predictions of global climate change models. Our results show a variety of effects of precipitation distribution, with the size of precipitation events being important at the driest site considered, and the spacing of events important at the mesic site. Differences between sites were greater than differences within sites created by changes in species composition and soil fertility. Clearly this generalisation needs to be tested elsewhere (Knapp *et al.* 2004). Such studies can only improve our understanding of the determinants of ANPP, and allow for more robust predictions of ecosystem responses to global climate change.

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TABLES AND FIGURES

Table 2.1. Summarized data for precipitation and ANPP[1] for each of the treatments analyzed at Bloemfontein, Towoomba and Ukulinga. At Bloemfontein both precipitation and ANPP data encompass 29 years, at Towoomba precipitation data are for 38 years and ANPP data are for 27 years, and at Ukulinga precipitation data span 49 years and ANPP data are for 30 years.

	Bloemfontein		**Towoomba**		**Ukulinga**	
Mean annual precipitation (mm)	538		645		798	
Mean % precipitation in summer	90		96		91	
CV of summer precipitation[2] (%)	35		27		21	
	Control	Poor	Control	P2N3	Control	N3P
Basal cover[3] (%)	9.0	3.4	12.6	9.8	15.8	-
Mean ANPP (g/m^2)	138	43	137	519	323	562
CV of ANPP (%)	53	58	55	65	26	43
Mean RUE[4] (g/m^2/mm)	0.25	0.08	0.21	0.77	0.48	0.80

[1] ANPP refers to total graminoid biomass produced in a year (forbs are a negligible component of total herbaceous biomass at each site).

[2] Based on precipitation data for growing seasons, up to cutting dates, for seasons for which ANPP were available.

[3] Basal cover data for Bloemfontein from Snyman (2000), measured from 1995 to 1999. For Towoomba value is the average of measurements from 1956 and 1982, from Donaldson *et al.* (1984). For Ukulinga control plot value is for adjacent plots subjected to the same cutting regime measured in 1997 (Morris & Fynn 2003); data not available for the fertilised treatment.

[4] RUE = rain use efficiency, calculated using annual precipitation.

Table 2.2. Linear regression models for the control and poor condition plots at Bloemfontein, and control and fertilized plots at Towoomba and Ukulinga. Model terms: AMOUNT = growing season precipitation total, INTERVAL = geometric mean of intervals between precipitation events, EVENT SIZE = geometric mean of precipitation event sizes, #EVENTS = number of precipitation events in a growing season. Parentheses give the standardized parameter estimate for each term. Terms in bold are significant at $p < 0.05$. Relative likelihood based on AICc. Outlier gives the largest DFFITS value.

Model terms	**r^2**	**Relative likelihood**	**Outlier**
BLOEMFONTEIN			
Control			
AMOUNT (0.68)	0.46	0.24	-3.07
EVENT SIZE (0.63) + #EVENTS (0.68)	0.68	1	<1
EVENT SIZE (0.38) + INTERVAL (-0.48)	0.48	0.06	-1.79
Poor condition			
AMOUNT (0.57)	0.32	0.93	-1.62
EVENT SIZE (0.62) + #EVENTS (0.45)	0.49	1	<1
EVENT SIZE (0.44) + INTERVAL (-0.36)	0.41	0.43	<1
TOWOOMBA			
Control			
AMOUNT (0.57)	0.32	1	<1
EVENT SIZE (0.62) + #EVENTS (0.27)	0.39	0.38	<1
EVENT SIZE (0.57) + INTERVAL (-0.04)	0.32	0.20	<1
EVENT SIZE (0.57)	0.32	0.997	<1
P2N3			
AMOUNT (0.85)	0.73	1	-1.03
EVENT SIZE (0.55) + #EVENTS (0.41)	0.38	<0.01	-2.32
EVENT SIZE (0.49) + INTERVAL (-0.24)	0.28	<0.01	-2.10
UKULINGA			
Control			
AMOUNT (0.47)	0.22	0.57	-1.92
EVENT SIZE (0.35) + #EVENTS (0.44)	0.26	0.15	<1
EVENT SIZE (0.40) + INTERVAL (-0.62)	0.44	1	<1
N3P			
AMOUNT (0.29)	0.08	0.41	-1.06
EVENT SIZE (0.20) + **#EVENTS (0.38)**	0.16	0.15	<1
EVENT SIZE (0.24) + **INTERVAL (-0.50)**	0.26	0.33	<1
INTERVAL (-0.45)	0.20	1	<1

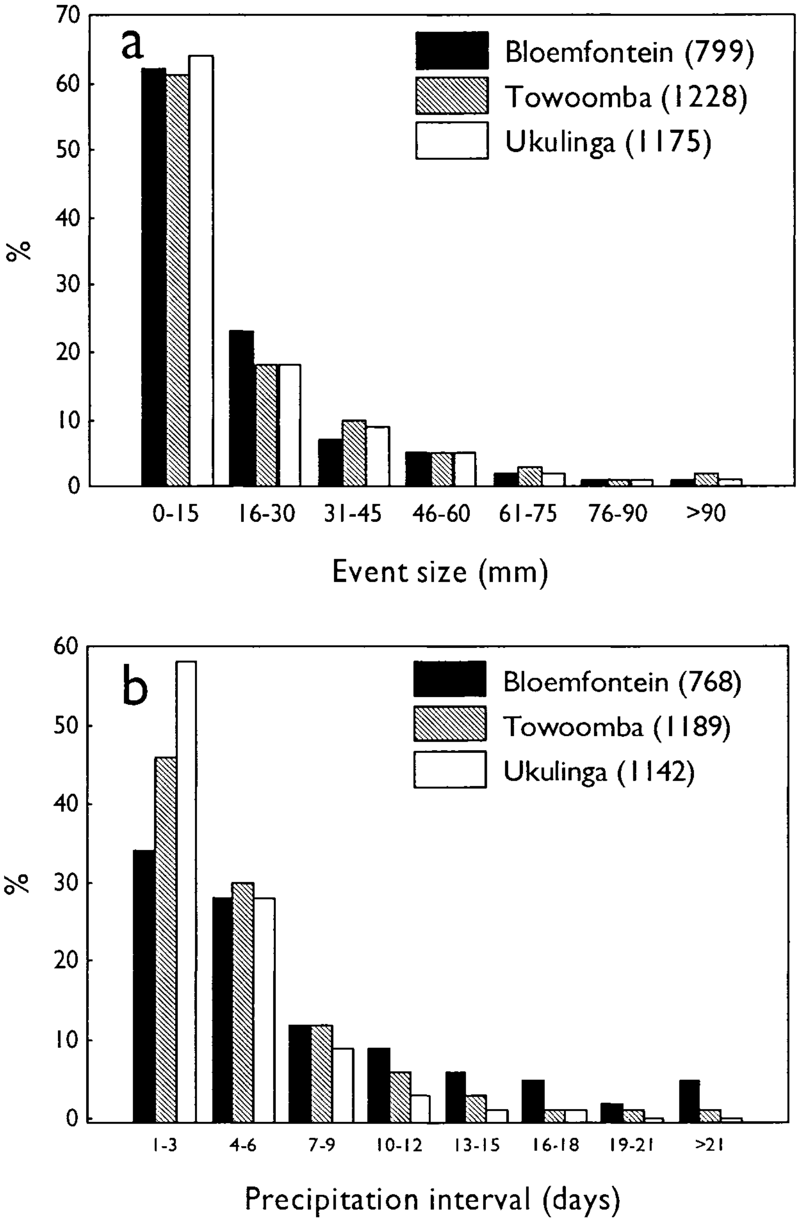

Figure 2.1. The distribution of a) precipitation events and b) intervals between precipitation events, for all growing-season data for Bloemfontein, Towoomba and Ukulinga. Data are untransformed values from growing seasons for which ANPP data were available, and are presented as percentages of all observations for each site (parentheses in legends show total observations). The size classes for each variable are arbitrary.

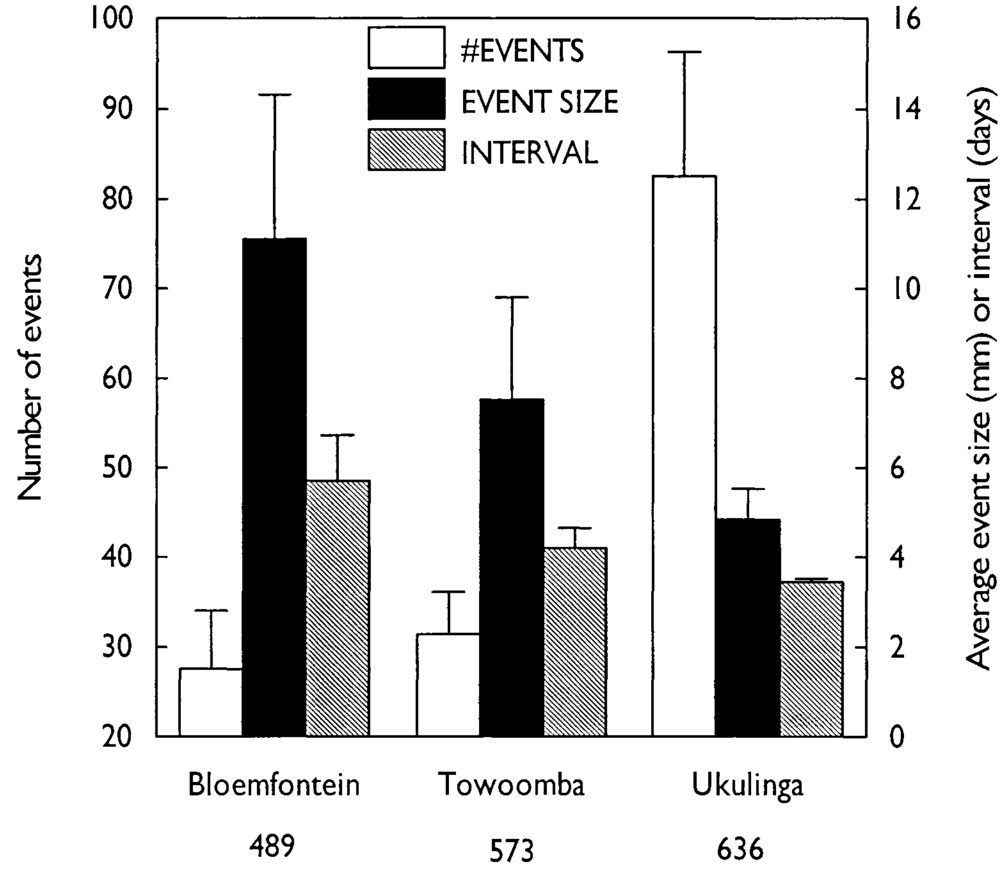

Figure 2.2. Mean number of precipitation events, average event size and average precipitation interval for all growing seasons for which ANPP data were analyzed for Bloemfontein (n = 29), Towoomba (n = 27) and Ukulinga (n = 30). For event size and precipitation interval, bars show the mean of each growing season average (each average being the geometric means for all events or intervals in a growing season). Values below each site are the mean seasonal precipitation (mm) for these seasons. Error bars show one SD. Refer to Table 2.2 for definitions of # EVENTS, EVENT SIZE and INTERVAL.

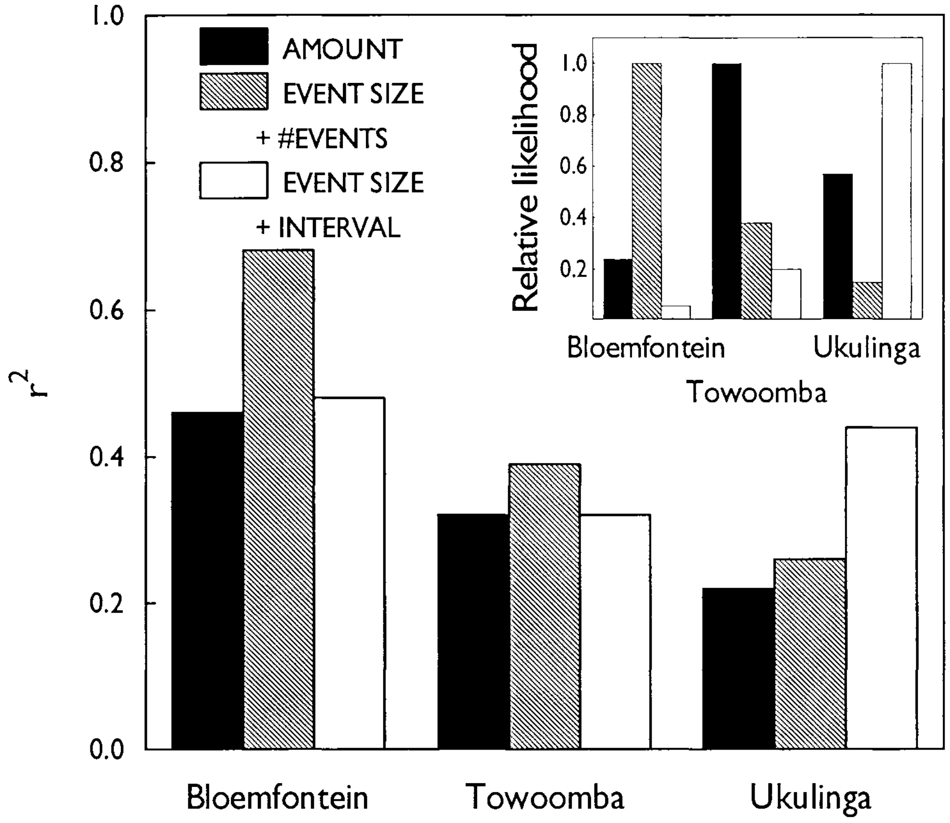

Figure 2.3. Regression coefficients for the three linear regression models fit to the control plot ANPP data for Bloemfontein, Towoomba and Ukulinga. Inset shows the relative likelihood value for each model, based on AICc. Refer to Table 2.2 for definitions of AMOUNT, # EVENTS, EVENT SIZE and INTERVAL.

CHAPTER 3

Defoliation synchronizes above-ground growth of co-occurring C_4 grass species

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ABSTRACT

The above-ground net primary productivity (ANPP) of grass communities in grasslands and savannas is primarily determined by annual precipitation. Recent research, motivated by predictions of changes in the distribution of rainfall events by global climate change models, indicate that ANPP may be affected by rainfall distribution during the growing season as much as by annual totals. Grazing and community composition can also have large effects of grassland ANPP, but little is known of how rainfall distribution, grazing and community composition may interact to control ANPP. The effects of community composition and grazing on above-ground growth responses to intra-seasonal variation in water availability were investigated with a non-selective clipping experiment, at seven grassland sites. The above-ground growth of the dominant C_4 species at each site was measured at regular intervals for 2-3 growing seasons, in the presence or absence of regular defoliation. In the absence of defoliation, there was a general lack of synchrony of intra-seasonal growth amongst co-occurring species. Variation in growth rates was generally high, but this variation could not be adequately explained by variation in rainfall. Regular defoliation increased synchrony at all sites, but changes in growth responses to rainfall varied between sites. These results suggest that grass community composition will be important in determining ANPP-precipitation relationships under conditions of altered rainfall distribution. However this effect appears to be more a result of species responding differently to soil water, or other resources, rather than rainfall *per se*. Non-selective grazing is predicted to over-ride the effects of community composition, and could uncouple ANPP from precipitation in some systems.

INTRODUCTION

The above-ground net primary productivity (ANPP) of grasslands and savannas is primarily determined by precipitation, and long-term data show a significant linear effect of annual precipitation on annual ANPP for sites around the world (Smoliak 1986, Lauenroth & Sala 1992, Briggs & Knapp 1995, Jobbagy & Sala 2000, O'Connor *et al.* 2001, Chidumayo 2003, McNaughton *et al.* 2003, Knapp *et al.* 2006, Nippert *et al.* 2006). Global climate change models predict not only changes in annual precipitation (Houghton *et al.* 2001) but also increases in "extreme events", with rainfall more likely to fall in larger but less frequent events (Gordon *et al.* 1992, Easterling *et al.* 2000, Meehl *et al.* 2005). Previous research on the effects of such changes in the distribution of rainfall events indicate that this effect can rival effects of changes in rainfall amount even in mesic grasslands (Knapp *et al.* 2002, Swemmer & Knapp 2007).

In addition to precipitation, herbivory and plant community composition can affect ANPP substantially. While these determinants have received less attention than precipitation, both grazing by ungulates (often simulated by clipping and mowing) and grass community composition have been shown to alter productivity-precipitation relationships at an annual time-scale (grazing: Coughenour 1991, Heitschmidt *et al.* 1999, Biondini *et al.* 2001, Frank *et al.* 2002, Chidumayo 2003; community composition: Kelly & Walker 1976, O'Connor *et al.* 2001). Grazing and community composition are also likely to affect ANPP responses to rainfall distribution, as the ability of individuals to exploit (or endure) intra-seasonal variation in the availability of water may depend on whether they have recently been defoliated, and on the genotypes present. For example, co-occurring grass species often reach peak biomass at different times of the season (Grunow *et al.* 1980, Sala *et al.*1981, Dye & Walker 1987) which may be a result of species-specific differences in responses to intra-seasonal variation in rainfall.

Interactions between these determinants of ANPP are made more complex by the potential for feedbacks between them. Inter-annual variation in precipitation has been shown to alter community composition (Snyman & Opperman 1983, Gibson & Hulbert 1987, O'Connor 1991) - particularly during drought (Weaver 1954, Herbel *et al.*1972, Snyman & van Resnburg 1990, Fynn & O'Connor 2000) - and these compositional changes may then alter the relationship between ANPP and annual precipitation. Likewise, substantial changes in community composition in response to grazing can occur (Snyman & Opperman 1983, O'Connor 1991, Collins & Steinauer 1998, Fynn & O'Connor 2000, Bullock *et al.* 2001). Predicting the response of ANPP of grass-dominated ecosystems to climate change therefore requires an understanding of not only the effect of annual precipitation, but also the various potential interactions between annual precipitation, rainfall distribution, herbivory and community composition.

To investigate the potential effects of grazing and community composition on the relationship between above-ground productivity and rainfall distribution, we conducted a defoliation experiment at a range of grassland sites, both semi-arid and mesic. The short-term, above-ground growth responses of co-occurring grass species was measured in both the presence and absence of defoliation, during intervals of high and low rainfall. In each treatment, the synchrony of intra-seasonal variation in growth provided a simple test of differences in species responses to intra-seasonal variation in rainfall: if all species respond in a similar way (the null hypothesis) then they should all grow at the same time (i.e. when water availability is low all species should show low growth rates, and when water availability increases they should show high growth rates). In contrast, a lack of synchrony could indicate that responses are species-specific. Regular defoliation was expected to disrupt any synchrony, as co-occurring species are known to vary in terms of regrowth following defoliation (McNaughton & Chapin 1985, Wallace *et al.* 1985, Klink 1994, Banyikwa 1998), and those less tolerant to defoliation were expected to become less responsive to intra-seasonal variation in water availability.

METHODS

Sites

Data were collected from seven sites located in pristine, un-ploughed perennial grasslands or savannas dominated by C_4 perennial grasses. Two of the sites were located in the USA: one in a mesic grassland in eastern Kansas and one in a semi-arid grassland in central Colorado. The remaining sites were located in South Africa: three in savannas within the Kruger National Park and two in grasslands within the Suikerbosrand Nature Reserve. Table 3.1 summarizes the main environmental conditions at the sites. All of the sites are fenced and ungrazed, and with the exception of Konza, had a long history of grazing prior to the start of the experiment.

Experimental design

At each site, the four most common C_4 grass species were selected for sampling. All except one were perennial. Six of the 28 species selected occurred at more than one of the sites. Two pairs of sites had two of these species, while a different two pairs of sites each shared one species. For all analyses, these overlapping species were treated as separate species at each site. Individual tufts were clipped at regular intervals (17 to 50 days) during the growing season, and all clipped material dried to a constant mass and weighed. Sampling intervals were deliberately shortened during wet periods when growth was more rapid in an attempt to measure a similar amount of growth in each interval. A defoliation and control treatment were established at each site with two plots per treatment. Each defoliation plot was located adjacent to a control plot. The proximity of each pair of defoliation and control plots varied from adjacent (Basalts and Sands) to approximately 100m (Konza). Defoliation treatment plot sizes varied from 3m x3m (Basalts and Sands) to 5m x 5m (Konza). Control plots were larger, extending away from the adjacent clip plots as far as was required to locate sufficient numbers of individuals to sample. None of the plots contained large trees or were shaded by canopies of nearby trees. Seven or eight individuals per species were typically clipped per plot at each sampling interval.

In the defoliation treatment randomly selected tufts were marked at the start of the experiment and clipped to a height of 2 - 3cm at the end of each interval. Clipped biomass therefore consisted of regrowth since the previous harvest. Individuals were marked and labeled using a plastic-coated loop of wire placed around the base of the tuft. After the marked tufts were clipped at the end of an interval, all other grass tufts and forbs in the plots were clipped down to the same height and this biomass removed from the plot. If a marked individual died, a replacement was selected from the same plot and marked, thus maintaining a constant number of replicates per plot (for some of the less common species, a shortage of additional individuals resulted in a reduction in replicates by the end of the experiment).

In the control treatment, individuals that had not been clipped previously were randomly selected at the end of each interval. These were then clipped down to the crown and marked with a flag to ensure they would not be clipped again. For strongly rhizomatous species, an individual was defined as a cluster of tillers that appeared to originate from the same rhizome. A new pair of control plots was established for each growing season and, in most cases, all plant material clipped to crown height prior to the onset of a season's growth. Thus all clipped material in the control treatment consisted of the current season's growth. For one season at the Sands and Basalts sites, and for all three seasons at Nkuhlu, control plots were neither burnt nor clipped down prior to the start of the growing season. Material harvested in these seasons was separated into senesced material from the previous season and material from the current season (on the basis on the color of senesced leaves) and the former discarded.

Sampling was conducted for three growing seasons (between September 2003 and July 2006). Due to logistical constraints, the following periods could not be sampled: the second half of the first growing season at the Basalts and Sands sites, the control plots at Satara, and for three species in the control plots at Shabeni. Similarly, for the second half of the third growing season, sampling was not possible at Konza and Boulder. These represented no more than 15% of the possible measurements that could have been made over the three year study period.

Precipitation measurement

Hourly precipitation data was obtained from automatic rain gauges. At the five South African sites these were located on site. For Boulder, data were obtained from a weather station of the National Wind Technology Center (www.nrel.gov/midc/nwtc_m2), approximately 3.5km from the site and at approximately the same altitude. For Konza, data were obtained from an automatic rain gauge located approximately 800m from the site (in watershed 4B). For a few intervals at some of the SA sites, data from the nearby weather station was used to substitute for missing data.

Statistical analysis

In the control treatment, growth was calculated as the increment in average biomass (live + dead) for each interval. t-tests of ln-transformed data, for each species in each interval, revealed no significant difference ($p = 0.05$) between the mean size of individuals in each control plot for the majority of intervals. Data from each plot were therefore combined to achieve a more accurate estimate of average biomass for each interval. Average biomass was calculated as the geometric mean because distributions were generally skewed to the right (using median biomass gave very similar results). For the defoliation treatment, the average growth for each species was calculated simply as the geometric mean biomass of all individuals at each sampling interval. Again replicates from both plots were combined. For the first interval, data for the defoliation plots was treated as control data, as individuals in these plots had not been clipped previously. Pair-wise correlations of growth for each species were calculated (using Pearson's product-moment) for each treatment at each site to assess the synchrony of growth (using intervals as replicates). Sample sizes ranged from nine to 18 in the control treatment and nine to 17 in the defoliation treatment. The pair-wise correlation coefficients from both treatments were then entered into a factorial ANOVA to test the effects of defoliation and site. To account for differences in sample sizes used to calculate the coefficients, each was weighted by its respective sample size.

To analyze species' growth responses to rainfall, growth rates were calculated as growth / interval length (days) to account for differences in interval lengths. Interval rainfall was calculated as the total rainfall for an interval (any rain that fell in the last three days of an interval was excluded and included in the following interval's total). Pearson's product-moment correlations of growth rate versus rainfall were calculated for each species in each treatment. For the control treatment this was simply done with a single correlation for each species. For the defoliation treatment, the effect of repeated measurements was accounted for with separate correlations for each marked individual (the biomass of each individual was divided by interval length to obtain individual growth rates for each interval). The least squares mean coefficient was then calculated for each species, using a factorial ANOVA with species and plot as the independent variables. As a result of some individuals dying, and being replaced, during the course of the experiment, many individuals were not measured for the entire experimental period, and therefore did not experience the full range of water availability encountered. To account for this in the ANOVA, each r value was weighted by the range of interval rainfall that the individual experienced. Individuals with less than three measurements were excluded, and the maximum sample size was 18.

For the control treatment, it was observed that many species reached peak biomass and stopped growing well before the end of the growing season. To account for this potentially confounding effect on growth-rainfall relationships, multiple regressions were performed for each species using both interval rainfall and plant size (the average biomass of a species at the start of an interval). Plant size was considered important if the additive or interaction effect of plant size reduced the Mallow's Cp statistic when added to a regression of growth rate versus interval rainfall. Plant size and interval rainfall were never strongly correlated, and the variance inflation factor was never greater than 5 for models that included plant size. A factorial ANOVA was used to test for the effects of defoliation, site and plant size on growth responses to rainfall, i.e. on the growth-rainfall correlation coefficients. All analyses were conducted using Statistica v7.0 (Statsoft Inc., Tulsa, USA). For the ANOVA's homogeneity of variance was checked using Cochran's C test, and the distribution of residuals with half-normal probability plots.

RESULTS

Synchrony

Variation in growth rate from one interval to the next was large for most species, but was greatly reduced by regular defoliation for almost all 28 species (Fig. 3.1). Synchrony of above-ground growth, measured as the strength of the correlation of interval growth between pairs of species, was generally weak in the control treatment (Fig. 3.2), but increased with regular defoliation at all seven sites. A weak interaction of defoliation and site (Table 3.2) resulted from smaller increases in synchrony at the two USA sites (Boulder and Konza) and one of the South African sites (Satara).

Rainfall correlations

Correlation coefficients of interval growth rate with rainfall during that interval varied greatly amongst species, as did the effect of regular defoliation on these correlations (Fig 3). Synchrony coefficients for pairs of species were lower for pairs that had a larger difference between the growth-rainfall coefficient for each species. Thus the greater the difference in growth responses to rainfall for a pair of co-occurring species, the weaker the synchrony of their growth rates. This negative relationship was stronger in the control treatment ($r = -0.40$, $p < 0.01$) than in the defoliation treatment ($r = -0.11$, $p = 0.50$), indicating that the greater synchrony in the defoliation treatment was not a result of uniformly stronger growth responses to rainfall amongst co-occurring species.

The strength of the correlations between growth rate and rainfall were dependent on site (Table 3). There was a significant interaction of the defoliation treatment and site, with correlations being consistently stronger in the defoliation treatment at two sites (Sands and Satara), but weaker at a third (Konza; Fig 3 – defoliation treatment differences at these sites were significant at the 5% level, according to a LSD test).

Twelve of the 28 species showed an effect of plant size on growth-rainfall regressions, with between one and three species affected at each site. Across all sites, pairs of species in which both species were affected by plant size showed far less synchrony than pairs with only one species affected, or neither species affected (Fig 4). In the control treatment, species affected by plant size had a larger mean CV of growth rates (206% versus 149%; one-tailed t-test: $t_{26} = 1.90$, $p = 0.034$). Differences in growth-rainfall correlations between species affected or not affected by plant size, averaged across all sites, were not significant (mean r for species affected = 0.25, not affected = 0.31; $t_{26} = 0.58$, $p = 0.29$), and the ANOVA revealed that the effect of plant size on changes in growth-rainfall correlations with defoliation was relatively weak, and depended on site (Table 3).

DISCUSSION

In the absence of defoliation there was substantial variation in above-ground growth dynamics of common, co-occurring, C_4 grass species at a range of sites spanning two continents. This suggests that community composition is likely to be a key determinant of ANPP responses to changes in rainfall distribution, and rainfall distribution may in turn drive changes in community composition (feedback that could either enhance or mitigate future changes in precipitation, depending on the species involved). These results lend to support to studies showing that greater diversity leads to greater productivity in grasslands (Tilman *et al.* 1996, Hector *et al.* 1999). More diverse communities are likely to be more productive over a range of rainfall distribution patterns, either because they are more likely to contain species that can remain (or become) productive for a given rainfall distribution (a selection effect *sensu* Drake 2003) or because of niche complementarity (Tilman *et al.* 2001). While evidence of niche complementarity for nitrogen use has been found at one grassland site (Tilman *et al.* 1996), our results suggests that it may also result from differentiation of water use, i.e. co-occurring species utilize water at different times of the growing season, resulting in greater community-level efficiency of water use.

The general lack of synchrony in the control treatment could only be partly attributed to differences in rainfall responses between co-occurring species. In addition, the effect of plant size, i.e. growth slowing as individuals grew larger, did disrupt synchrony amongst some co-occurring species. This effect was evident for fast-growing species that typically grew rapidly following adequate rainfall early in the growing-season, but then ceased growth well before the end of the growing season, regardless of water availability (pers. obs.). This resulted in greater intra-seasonal variation in growth rates, and poor correlation of growth rates and interval rainfall for these species. The plant size effect could have arisen from of any number of growth constraints that develop during the growing season. Size itself may be important, as self-shading can be important in grasses (Knapp & Seastedt 1986). Other factors include phenology (some species appeared to stop growing once they had flowered), competition (greater shading by neighbours) or declining soil nutrient availability. For two species, growth rates actually increased with "plant size" suggesting an exponential pattern of growth typically observed under optimum conditions. Even when the effect of plant size was accounted for, the large variation in interval growth rates was only weakly associated with interval rainfall for many species, as was the case for many species unaffected by plant size. Plants respond to soil water availability, rather than rainfall *per se*, and at the temporal scale of this study rainfall and soil water availability may not be strongly correlated due to storage of water in deeper soil layers (Heitschmidt *et al.* 1999, 1969 Sala *et al.* 1992, Paruelo & Sala 1995, Snyman 2000). Therefore it is likely that intra-seasonal variation in soil water content would be a better predictor of short-term growth rates than recent rainfall. Furthermore, deep-rooted species may be responding to soil water content at a particular depth (e.g. Holmes & Rice 1996), and the role of community composition in determining ANPP responses to altered rainfall regimes may therefore depend on how intra-seasonal variation in rainfall translates into both temporal and spatial variation in soil water availability.

Regular defoliation clearly reduced intra-seasonal variation in above-ground growth, both within and between species, and increased synchrony among co-occurring species. This suggests that ANPP responses to altered rainfall distributions will be less complex in heavily-grazed systems. Regular, non-selective clipping created redundancy amongst species, and changes in community composition in heavily-grazed environments (resulting from the grazing or other factors) are therefore predicted to be less important for determining ANPP-precipitation relationships. The greater synchrony created by regular defoliation was not necessarily a result of stronger growth responses to rainfall amongst co-occurring species. While growth responses to rainfall were stronger in the defoliation treatment for some species, they were weaker for others, with site-specific factors having an over-riding effect. Rather than creating uniform responses to rainfall, the strongest effect of regular clipping appeared to be to constrain the growth patterns of the fast-growing, high-variance species to those of the more consistent, slower-growing species, whose short-term growth was little affected by large rainfall events. This suggests that, for certain systems, the major effect of regular, non-selective grazing may simply be to uncouple ANPP from annual precipitation, with the rapid growth responses to rainfall precluded for all species, and ANPP constrained to low levels even in wet years.

Regular defoliation also effectively lengthened the growing season for many species. The removal of the constraint of plant size resulted in all species producing some growth (following clipping) even at the end of the growing season, as has been found for tillering (Butler & Briske 1998) and green leaf duration (Baruch & Bilbao 1999). These results have important implications for the co-evolution of grasses and grazers. By returning to the same patch to feed, grazers may incur a cost of a lower quantity of forage but benefit from a more consistent supply, with at least some regrowth available for consumption for the entire length of the growing season.

Finally, while the clipping treatment in this experiment simulated non-selective grazing, the effects of selective grazing are likely to add complexity to ecosystem responses to climate change. Selective grazing can lead to a rapid decline of palatable species (Anderson & Briske 1995), which could strengthen or weaken ANPP-precipitation relationships, depending on the rainfall responses of the unpalatable species that become dominant.

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TABLES AND FIGURES

Table 3.1. Locations and environmental variables for the seven sites where the experiment was conducted. “MAP” = mean annual precipitation (parentheses indicate the approximate percentage of precipitation that occurs during the growing season). “Rooting depth” refers to the approximate maximum grass root depth observed when installing soil probes. “Species richness” is the numbers of species (Poaceae) recorded within the study site (parentheses shows the number of these that are C_3).

Site	Location	Coordinates	MAP (mm)	Soils	Rooting depth (cm)	Summer temperatures (°C)	Burning history	Species richness
Basalts	Suikerbosrand Nature Reserve	26° 28.105’ S 28° 11.270’ E	665 (68%)[2]	Basalt derived. Clay-rich. Ferrocrete present.	55	mean max: 25 mean min: 13 [4]	Burnt every 3 years; burnt immediately prior to start of experiment and prior to 3rd season	14 (0)
Boulder	City of Boulder Open Space System, Van Vleet property	39° 53.583’ N 105° 14.700’ W	457 (55%)[3]	Sandstone derived glacial outwash. Clay loam, shallow, rocky. [3]	-	mean max: 23 mean min: 12 [3]	No previous burning but burnt prior to 3rd season of experiment.	13 (8)
Konza	Konza Biological Research Station, watershed 1B	39° 4.308’ N 96° 35.999’ W	835 (75%)[1]	Limestone and shale derived. Clay rich, deep. [6]	-	mean max: 27 [7] mean min: 16	Annually in spring.	7 (1)
Nkuhlu	Kruger National Park, Nkuhlu large mammal exclosure	24° 59.333’ S 31° 46.458’ E	550 (87%)[5]	Granite derived. Sandy.	60	mean max: 33 mean min: 18 [5]	Previous burning irregular; no burning since 2 years prior to experiment	22 (0)
Sands	Suikerbosrand Nature Reserve	26° 31.607’ S 28° 17.065’ E	665 (68%)[2]	Sandstone derived. Sandy.	60	mean max: 25 mean min: 13 [4]	Burnt every 3 years; burnt prior to start of experiment and prior to 3rd season	29 (0)
Satara	Kruger National Park, exclosure within the Satara buffalo camp	24° 40.486’ S 31° 74.756’ E	544 (88%)[5]	Basalt derived Clay-rich. Calcrete present	75	mean max: 32 mean min: 19 [5]	Burnt approximately every 3 years; no burning since 1 year prior to experiment	12 (0)
Shabeni	Kruger National Park, Exclosure on the Shabeni burn plots	25° 08.716’ S 31° 14.083’ E	737 (87%)[5]	Granite derived Sandy.	90	mean max: 31 mean min: 17 [5]	Previously burnt every 2 years. No burning since 1 year prior to experiment.	28 (0)

[1] Hayden (1998); [2] Reilly and Panagos (2002); [3] City of Boulder Open Space and Mountain Parks (1986); [4] South African Weather service data Johannesburg , 1961-1990 (www.weathersa.co.za); ; [5] Nick Zambatis, Scientific Services, Kruger National Park, 1950 - 1990; [6] Ransom et al. (1998); [7] Fay et al. (2003)

Table 3.2. The effect of site and defoliation treatment on above-ground growth synchrony. A factorial ANOVA was conducted using growth correlations for the six pairs of species at each site.

Effect	SS	df	F	P
Defoliation	37.9	1	62.6	<0.01
Site	7.4	6	2.0	0.07
Defoliation x Site	6.4	6	1.8	0.12
Error	42.4	70		

Table 3.3. The effects of site, defoliation treatment and the effect of plant size on growth in the control treatment (Size), on the growth responses to rainfall. A factorial ANOVA was conducted using the interval growth versus rainfall correlation coefficients for all 28 species.

Effect	SS	df	F	p
Defoliation	0.004	1	0.19	0.67
Site	1.34	6	9.50	<0.01
Size	0.04	1	1.56	0.22
Defoliation x Site	0.87	6	6.14	<0.01
Defoliation x Size	0.001	1	0.01	0.92
Site x Size	0.38	6	2.73	0.03
Defoliation x Site x Size	0.33	6	2.34	0.06
Error	0.66	28		

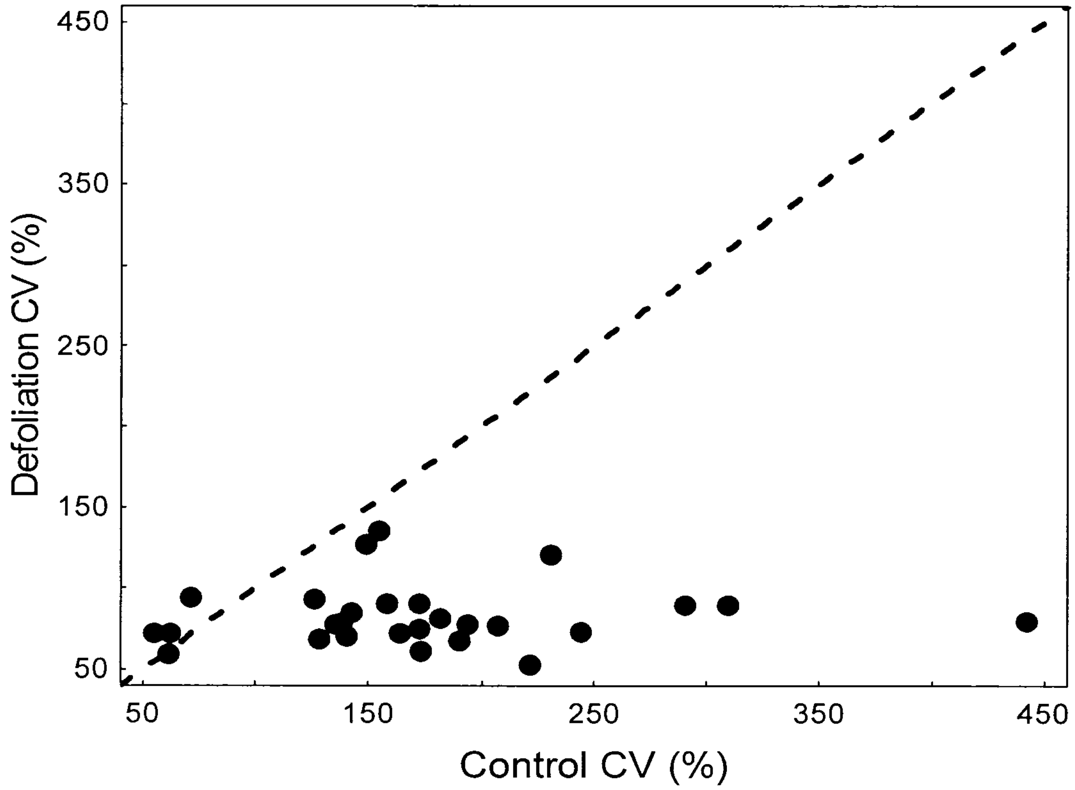

Figure 3.1. The coefficient of variation (CV) of growth rates in the control and defoliation treatments calculated from the 9-18 intervals sampled across the seven sites. Each point represents the CV for one of the 28 species in each treatment. The dashed line shows unity. Defoliation thus decreased the CV for almost all species.

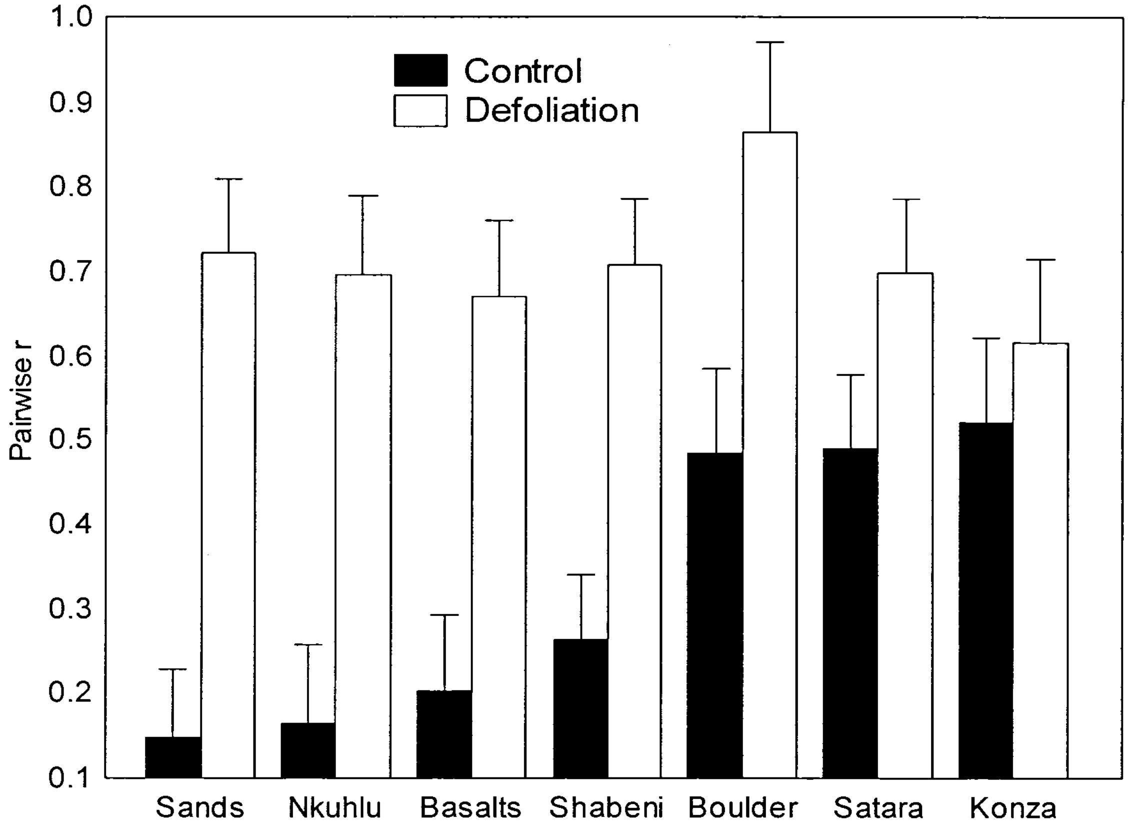

Figure 3.2. Mean correlation coefficients for growth between the six pair-wise comparisons of the four species at each site. Data are shown for both control and defoliated treatments. For the calculation of the mean coefficient for each treatment at each site, the pair-wise coefficients were weighted by the number of intervals of measurement for each pair of species at the site. Error bars show 1 SE. Refer to Table 2 for ANOVA results.

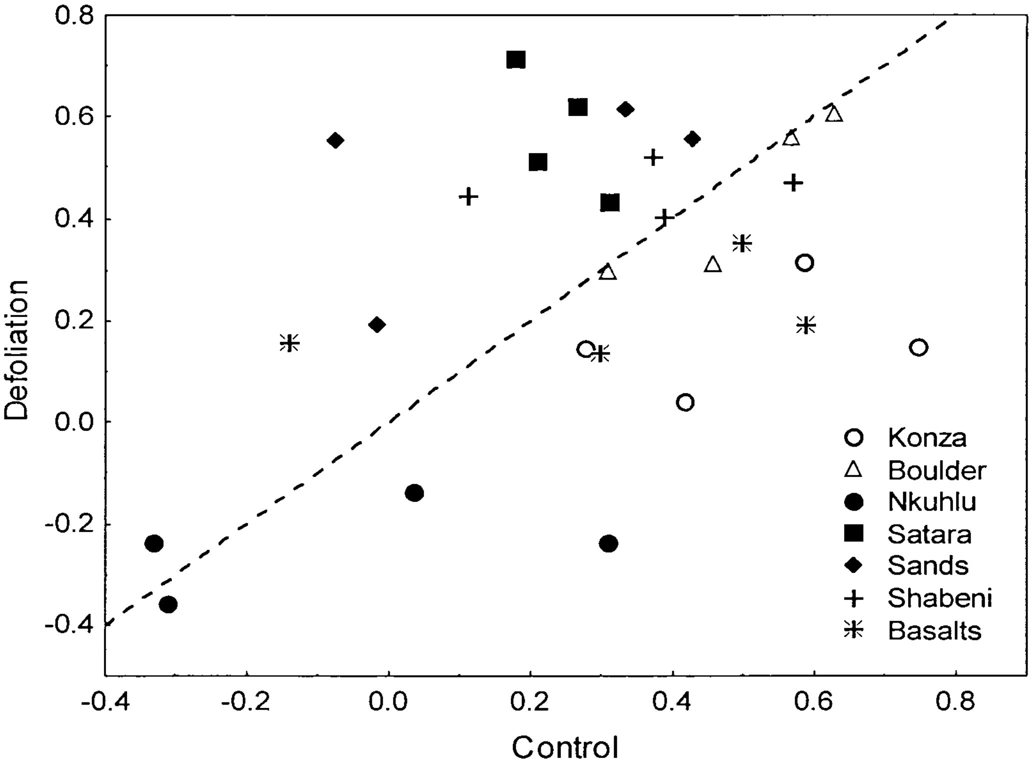

Figure 3.3. Correlation coefficients for growth versus rainfall, plotted for the control and defoliation treatments, for each species. Species are labeled according to site. The dashed line shows unity.

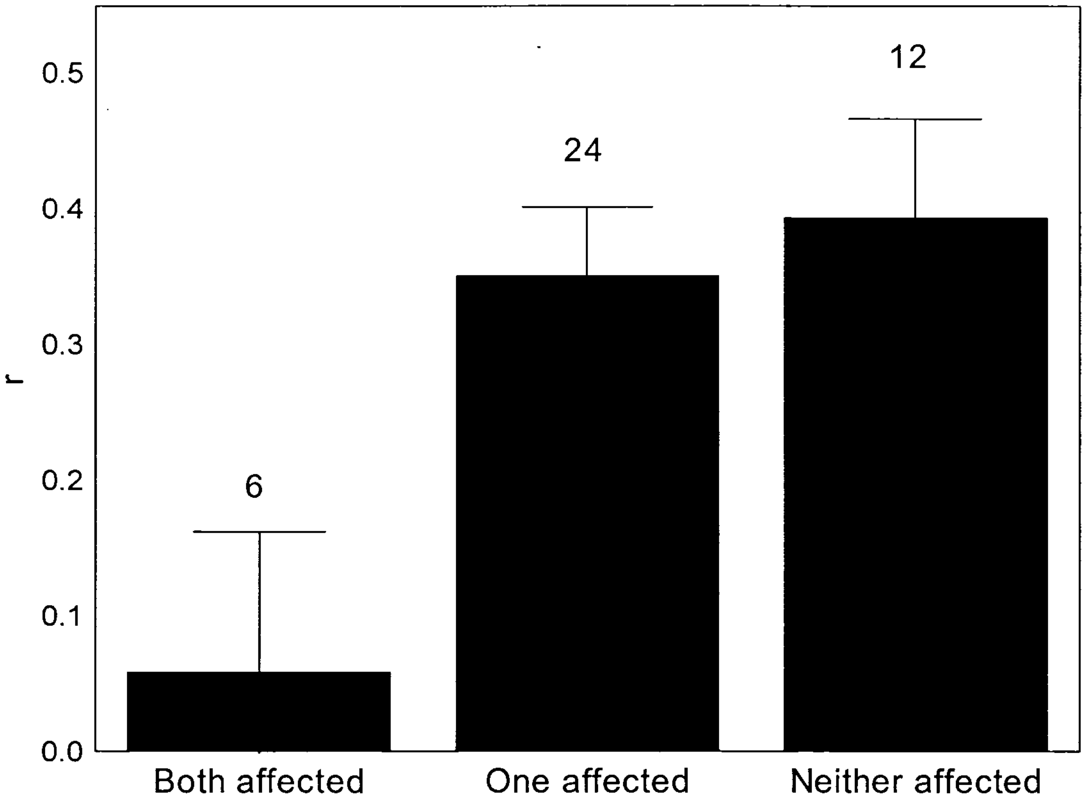

Figure 3.4. The mean coefficient of pair-wise growth correlations for pairs of species grouped according to the effect of plant size on growth-rainfall regressions. A one-way ANOVA indicated a significant effect of the grouping ($F_{2,39} = 3.9$, $p = 0.03$). Error bars show 1 SE. Numbers above the bars are the number of species pairs in each group.

CHAPTER 4

Above-ground growth responses to soil water and defoliation differ amongst co-occurring C_4 grass species

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ABSTRACT

Precipitation is generally accepted as the primary determinant of annual above-ground net productivity (ANPP) in grasslands. Long-term studies showing the response of ANPP to annual precipitation do not generally include any interacting effects of grass community composition or grazing, although both these factors are known to affect ANPP independently. Annual precipitation and the distribution of precipitation within the growing season, both of which are predicted to change by global climate change models, may themselves drive changes in community composition. To determine the potential effect of community composition on ANPP-precipitation relationships, the above-ground growth of common, co-occurring grass species was measured at regular intervals within the growing season, for 2-3 growing seasons at five sites. Intra-seasonal growth rates were as regressed against average soil water content measured at three different depths. To determine any effects of grazing, certain individuals were clipped repeatedly. In the absence of regular defoliation, vertical partitioning of soil water was evident, with co-occurring species primarily utilizing either near-surface water or deeper water closer to the base of the rooting zone. Regular defoliation resulted in a convergence towards shallow water use at four out of five sites. These results suggest that changes in community composition may be driven by changes in precipitation patterns, as a result of species-specific responses to temporal variation of soil water at different depths. In addition, changes in grass species composition are likely to alter the relationship between ANPP and precipitation in the systems studied, while non-selective grazing is predicted to mitigate this effect.

INTRODUCTION

Precipitation is an important determinant of above-ground net primary productivity (ANPP), particularly in grasslands where long-term data show a significant linear effect of annual precipitation on annual ANPP for a range of sites (Smoliak 1986, Lauenroth & Sala 1992, Briggs & Knapp 1995, Jobbagy & Sala 2000, O'Connor *et al.* 2001, Chidumayo 2003, McNaughton *et al.* 2003, Knapp *et al.* 2006, Nippert *et al.* 2006). These studies provide insights into the determinants of ANPP, and may be useful for predicting ANPP in response to changes in precipitation regimes forecast by global climate change models (Houghton *et al.* 2001). However, they usually involve plant communities that are ungrazed, and have relatively constant species composition. As most grasslands experience some degree of grazing, and as changes in community composition can substantially alter productivity-precipitation relationships (Kelly and Walker 1976, O'Connor *et al.* 2001, Suttle *et al.* 2007, Swemmer *et al.* 2007), the generality of the results of these long-term studies is limited.

Changes in community composition can occur in response to grazing (Snyman & Opperman 1983, Collins & Steinauer 1998, Hartnett & Fay 1998, Sternberg *et al.* 2000, Bullock *et al.* 2001), fire (Collins & Steinauer 1998, Snyman 2003), fertilization (Donaldson & Rootman 1984, Fynn & O'Connor 2005) and inter-annual variation in precipitation (Gibson & Hulbert 1987), particularly during drought (Herbel *et al.* 1972, Weaver 1954). Compositional changes resulting from the latter could create feedbacks that either reduce or intensify the effects of future changes in precipitation regimes on grassland productivity. The degree to which changes in composition alter ANPP-precipitation relationships would depend on the degree to which the productivity-precipitation relationships of the dominant and potentially dominant species differ. Intra-seasonal variation in precipitation may be particularly important in this regard. The size and distribution of rainfall events can vary greatly within a growing season in grasslands (Swemmer *et al.* 2007), and observations of co-occurring grass species reaching their peak biomass at different times of the season (Dye & Walker 1987, Grunow *et al.* 1980, Sala *et al.* 1981, Swemmer & Knapp 2007) may be result of different species responding to intra-seasonal variation in rainfall. Dye & Walker (1987) have suggested that erratic rainfall patterns are responsible for different C_4 grasses species dominating a semi-arid savanna in different years. Furthermore, global climate change models predict substantial changes in the distribution of precipitation events (Easterling *et al.* 2000, Gordon *et al.* 1992, Meehl *et al.* 2005) for many of the grassland and savanna regions of the world.

Differences in the distribution of rainfall within the growing season would create differences in the temporal patterns of water availability at different depths in the soil profile, depending on site specific factors such as soil texture and depth. Co-occurring species are therefore likely to differ in productivity-precipitation relationships if they differ in terms of depth of soil water uptake and / or the ability to tolerate temporal variation in water availability. In addition, as regular defoliation can have a large impact on both the above-ground (Chapin & McNaughton 1989, Chidumayo 2003, Coughenour *et al.* 1985a,b, Kotanen & Bergelson 2000, Oesterheld & McNaughton 1988, Schmitt 1997, Wallace *et al.* 1985, Wand *et al.* Midgley 2002) and below-ground (Dawson *et al.*

2000, Jaramillo & Detling 1988, Peaz *et al.* 1995, Ruess *et al.* 1983) growth of grasses, grazing could substantially alter the effects of compositional changes on ANPP-precipitation relationships.

In this study, we investigated differences in the above-ground growth responses of co-occurring native, perennial C_4 species to intra-seasonal variation in soil water content at different depths. A defoliation treatment was included to determine the potential effects of grazing of such differences.

METHODS

Sites

Data were collected from five sites located in pristine, un-ploughed perennial grasslands or savannas dominated by C_4 perennial grasses. All the sites were located in South Africa: three in savannas in a low-lying semi-arid region, and two in mesic grasslands in a high-lying region. Table 4.1 summarizes the main environmental conditions at the sites. All of the sites were fenced and ungrazed for the duration of the experiment, but all had a long history of grazing prior to the start of the experiment.

Experimental design

At each site, the four most common C_4 grass species were selected for sampling. All except one were perennial. Four of the 20 species selected occurred at two different sites, resulting in one pairs of sites having two species in common, and another two pairs of sites having one species in common. The overlapping species were treated as separate species at each site. Individual tufts were clipped at regular intervals (17 to 50 days) during the growing season, and all clipped material dried to a constant mass and weighed. Sampling intervals were deliberately shortened during wet periods when growth was more rapid in an attempt to measure a similar amount of growth in each interval. A defoliation and control treatment were established at each site with two plots per treatment. Each defoliation plot was located adjacent to a control plot. The proximity of each pair of defoliation and control plots varied from adjacent (Basalts and Sands) to approximately 50m (Shabeni and Satara). Defoliation treatment plot sizes varied from 3m x 3m (Basalts and Sands) to 4m x 4m. Control plots were larger, extending away from the adjacent clip plots as far as was required to locate sufficient numbers of individuals to sample. None of the plots contained large trees or were shaded by canopies of nearby trees. Seven or eight individuals per species were typically clipped per plot at each sampling interval.

In the defoliation treatment randomly selected tufts were marked at the start of the experiment and clipped to a height of 2 - 3cm at the end of each interval. Clipped biomass therefore consisted only of regrowth since the previous harvest. Individuals were marked and labeled using a plastic-coated loop of wire placed around the base of the tuft. After the marked tufts were clipped at the end of an interval, all other grass tufts and forbs in the plots were clipped down to the same height and this biomass removed from the plot. If a marked individual died, a replacement was selected from the same plot, thus maintaining a constant number of replicates per plot (for some of the less common species, a shortage of additional individuals resulted in a reduction in replicates by the end of the experiment).

In the control treatment, individuals that had not been clipped previously were randomly selected at the end of each interval. These were then clipped down to the crown and marked with a flag to ensure they would not be clipped again. A new pair of control plots was established for each growing season and, in most cases, all plant material clipped to crown height prior to the onset of a season's growth. Thus all clipped material in the control treatment consisted only of the current season's growth. For one season at the Sands and Basalts sites, and for all three seasons at Nkuhlu, control plots were neither burnt nor clipped down prior to the start of the growing season. Material harvested in these seasons was separated into senesced material from the previous season and material from the current season (on the basis on the color of senesced leaves) and the former discarded.

Sampling was conducted for three growing seasons (between September 2003 and July 2006). Due to logistical constraints, sampling could not be conducted for the second half of the first growing season at the Basalts and Sands sites, in the control plots at Satara, and for three species in the control plots at Shabeni. These represented less than 13% of the possible measurements that could have been made at any of the sites over the three year study period.

Soil water measurement

Volumetric soil water content was measured at three to four depths in the center of in each plot using 20cm capacitance probes (Echo probes, Decagon Devices, Inc.). The probes were buried horizontally at 6.5cm (referred to as depth A), at the base of the A horizon (25 to 30cm deep, depending on the site; depth B) and at the base of the rooting zone, usually the base of the B horizon (55 to 75cm deep, depending of the site; depth C). At Shabeni, soils were particularly deep and the probe at depth C was about 30cm above the base of the B horizon. An additional probe was buried at the base of the B horizon, at a depth of 90cm, in two of the plots at this site. Thermisters (Decagon Devices, Inc.) were buried alongside the A depth probes in all plots, as well as some of the B depth probes in certain plots. Output from all probes was logged hourly. Separate calibrations were performed for the capacitance probes using soil from the A and B horizon at each site following the method recommended by the manufacturers (Campbell 2002). As diurnal temperature fluctuations appeared to affect the capacitance probe output, temperate effects were included in the calibration by heating the calibration soil in an oven while simultaneously recording probe output and soil temperature (using two thermisters buried alongside the calibration probe). Temperature effects were subsequently included in the calibration equations for depth A for all sites except Sands, where no diurnal temperature fluctuations were evident. Diurnal fluctuations were not evident for probes at the B and C depths at any site.

Hourly volumetric water content values for each depth of measurement were averaged for both plots in each treatment, and the mean of these values used as a measure of average interval soil moisture for a particular depth (if a datum for one plot was missing, the datum for the other plot for that treatment was used in place of an average). For the Shabeni site, data from the probes buried deeper than depth C was highly correlated with the data from the latter and produced very similar results in the growth rate analyses (below).

Statistical analysis

To describe the temporal variability of soil water, the hourly data for each depth were averaged, and the coefficient of variation (CV) of these daily mean values calculated for each interval, for each depth in each treatment at each site. The mean of these interval CV values was used as a measure of within-interval variation. To describe variability between intervals, the hourly means were averaged to create an overall mean water content for each interval, and the CV of these means was then calculated for each depth, in each treatment at each site.

In the control treatment, growth was calculated as the increment in average biomass (live + dead) for each interval. t-tests of ln-transformed data, for each species in each interval, revealed no significant difference ($p = 0.05$) between the mean size of individuals in each control plot for the majority of intervals. Data from each plot were therefore combined to achieve a more accurate estimate of average biomass for each interval. Average biomass was calculated as the geometric mean because distributions were generally skewed to the right (using median biomass gave very similar results). For the defoliation treatment, the average growth for each species was calculated simply as the geometric mean biomass of all individuals at each sampling interval. Again replicates from both plots were combined. For the first interval, data for the defoliation plots was treated as control data, as individuals in these plots had not been clipped previously.

To analyze species' growth responses to water availability, growth rates were calculated as growth / interval length (days) to account for differences in interval lengths. In the control treatment, linear regressions of interval growth rate versus mean interval soil water content at depth A, B or C were used to determine the best predictor of growth (as soil water content was often correlated at the two adjacent depths, their relative effects could not be tested together in a multiple regression). The depth that accounted for the most variation in interval growth rates for each species is referred to as the "optimum depth" hereafter. An additional variable, "plant size" (the average biomass of a species at the start of an interval) was included in multiple regressions with the optimum depth for each species, as it was observed that some species stopped growing well before the end of the growing season if they had reached a certain size. This variable was thus used to account for some of the effects of factors other than soil water availability on growth rates. Plant size was considered important if its inclusion, as either an additive or interaction effect, reduced the Mallow's Cp statistic for the growth rate versus water content regression. This provided a conservative test of its effect. Water content and plant size were never strongly correlated, and the variance inflation factor was never greater than 5 for models containing both variables.

For the defoliation treatment, growth responses to water availability were calculated differently to account for the effect of repeated measurements. For each interval, the biomass of each individual was divided by interval length, and linear regressions of growth rates versus water content (at each depth) were performed separately for each individual. A mean r^2 value was then calculated for each species at each depth. As a result of some individuals dying, and being replaced, during the course of the experiment, many individuals did not experience the full range of water availability encountered during the entire experimental period. To account for this, each individual's r^2 value was weighted by the range of interval water contents that the respective individual experienced. Mean r^2 values were then calculated as least-square means (i.e. averaged by block first) to account for any differences in sample sizes between blocks. Individuals with less than three measurements were excluded. The largest weighted mean r^2 value for each species was used to select the optimum depth.

Log-linear analysis was performed to test for an effect of site and defoliation treatment on optimum depth. All analyses were performed using STATISTICA 7.0 (StatSoft Inc., Tulsa, USA).

RESULTS

Temporal variability of soil water content within sampling intervals was generally greater nearest the surface and declined with depth (Fig. 4.1). The large variation at the shallowest depth was the result of short-lived pulses in water content following rainfall events. At deeper depths, soil was often uniformly wet or dry within intervals. For depth A, variation of soil water content between intervals was similar or even lower than the variation within intervals, indicating that sampling was not frequent enough to capture the rapid temporal dynamics of water availability near the surface. Variation between intervals was greater than within intervals for depths B and C, indicating that sampling intervals were more uniformly wet or dry. Regular defoliation had no consistent effect on soil water variability at either temporal scale, with both increases and decreases evident at different depths and sites.

In the control treatment, growth rate regressions with soil water content differed widely between species, in terms the amount of variation in growth rates that could be explained, the depth which provided the strongest regression, and the effect of plant size. r^2 values ranged from 0.07 to 0.83 (Table 4.2), but did not differ significantly between sites (one-way ANOVA: $F_{4,15} = 0.89$, $p = 0.50$). Within each site there were at least two different optimum depths (Fig. 4.2). At two sites (Sands and Shabeni), all four species showed an effect of plant size, while at least one species showed such an effect at each of the remaining sites.

In the defoliation treatment, the growth rate regressions were generally weaker, with a maximum r^2 of 0.54 (Table 4.2), although r^2 values did not differ significantly for the seven species that showed no effect of plant size in the control treatment (t-test for paired samples: mean difference = 0.015, $t_7 = 0.17$, $p = 0.87$). A site effect was evident, as a result of higher r^2 values for the Satara species (one-way ANOVA: $F_{4,15} = 5.22$, $p < 0.01$). More co-occurring species had the same optimum depth than in the control treatment (Fig 4.2) - a result of eight species having a more shallow optimum depth in the defoliation treatment. Eleven species did not show a change in optimum depth while and one had a deeper depth. Species that changed to a shallower depth had marginally weaker regressions that those with the same or deeper optimum depth (mean r^2 of 0.24 versus 0.34; t-test: $t_{18} = 1.8$, $p = 0.08$).

Log-linear analysis indicated a close association of the observed and fitted optimum depth frequencies ($\chi^2 = 3.5$, df = 8, $p = 0.90$), indicating that both site and defoliation had a significant effect on the frequency of different optimum depths within sites. The site effect (partial association $\chi^2 = 35$, df = 8, $p < 0.01$) was the result of an absence of species with a particular optimum depth at four of the five sites. The weaker defoliation effect (partial association $\chi^2 = 8.9$, df = 2, $p = 0.01$) was the result of fewer species having an optimum depth C in the defoliation treatment than would be expected by chance.

DISCUSSION

Across all five sites, each differing in soil type or mean annual precipitation, common co-occurring grass species showed different growth responses to soil water availability at different depths. Certain species utilized primarily either shallow or deep soil water for the bulk of their above-ground growth, at least in the absence of regular defoliation, indicating vertical partitioning of soil water. While spatial differences in soil water utilization have previously been shown for trees versus grasses (McAuliffe 2002) and deep-rooted perennials versus shallow-rooted exotic annuals (Holmes & Rice 1996), they have only been inferred for perennial grasses, on the basis of differences in rooting depth (e.g. Weaver and Fitzpatrick 1932). The results contrast those of Fargione & Tilman (2005), who attributed differences in root depth distributions between perennial grasses to vertical partitioning of soil nitrate.

All the sites involved in this study constitute "empty bucket" systems (*sensu* Knapp *et al.* 2006), with soil water contents at minimum levels (down the entire soil profile) at the start of the growing season. All the species studied did appear to utilize at least some water in the shallow soil layers, as some growth was observed for all species early in the growing season, when soil water content at depth was always low. However, maximum growth rates of those species with deeper optimum depths only began towards the middle of the growing season, when sufficient rainfall had fallen to create infiltration to the deeper layers. In contrast species with a shallow optimum depth reached their peak biomass fairly early in the growing season, before any significant infiltration to depth occurred, revealing an ability to tolerate the large temporal variability of water supply in the upper soil and indicating an association between spatial and temporal partitioning of the soil water resource. The lack of utilization of the more stable supply of water in the deeper soil, later in the growing season, may have simply been the result of an inherent constraint on the allocation of assimilates below-ground that restricts rooting depths. Alternatively the growth of these species could have been constrained by other factors by the time that water content increased in the deeper layers. Such seasonal constraints were evident for some of the species with a shallow optimum depth, as indicated by an effect of plant size on growth rates. This effect may have been a result of self-shading, or other extrinsic factors that developed as the growing season progresses, such as phenology (some species appeared to stop growing once they had flowered), competition (e.g. greater shading by neighbours) or declining soil nutrient availability.

The observed differences in optimum depth between species did not depend on site-specific factors, as species utilizing primarily either shallow or deeper water co-occurred at all five sites. This apparent niche separation may be an important factor contributing to the co-existence of otherwise similar C_4 grass species in many grasslands and savannas. These results also provide evidence of niche complementarity, and are consistent with studies showing that greater diversity leads to greater productivity in grasslands (Tilman *et al.* 1996, Hector *et al.* 1999).

The functional diversity observed within sites suggests that changes in species composition could have an important effect on ANPP-precipitation relationships at the ecosystem level. The above-ground growth of species with a shallow optimum depth would be less affected during dry years, when there is little infiltration to deeper soil layers, than that of species with a deep optimum depth. The same may occur in years where long intervals between major rainfall events translate into less consistent water availability in the deeper layers. Conversely, in wet years species with shallow optimum depths would not be able to take advantage of the additional water reaching the deeper soil layers, and would likely suffer increased competition from deep optimum depth species. Changes in the relative abundance of species with either shallow or deep optimum depths would therefore alter rain-use efficiency in both years of below- and above-average precipitation. Over a longer time period, different growth responses of the shallow- and deep-optimum-depth species could feedback into population-level responses that alter production-precipitation relationships even in years with average precipitation.

Regular defoliation resulted in a convergence to a more shallow optimum depth at four of the five sites, as a result of certain species primarily utilizing soil water at a shallower depth. This convergence towards utilization of the same water source is consistent with the greater synchrony of intra-seasonal growth following regular defoliation, for the same species, reported by Swemmer & Knapp (2007). The weaker relationship between growth and water availability for those species that showed a change in optimum depth suggests that they were not as well adapted to utilizing the more variable supply of water at a shallower depth. Utilization of more shallow soil water is consistent with the results of simulation models (Coughenour 1984, Coughenour *et al.* 1984), pot studies (Dawson *et al.* 2000, Jaramillo & Detling 1988, Ruess *et al.* 1983) and field experiments (Pandey & Singh 1992, Richards 1984) showing reduced root growth following defoliation. Other studies, showing unchanged or even greater allocation to roots following defoliation (Coughenour *et al.* 1985a, Dawson *et al.* 2000, Kotanen & Bergelson 2000) or in heavily-grazed communities (Frank *et al.* 2002, McNaughton *et al.* 1998) are consistent with the result of no change in optimum depth for the other species.

The change in optimum depth for many species suggests that regular, non-selective grazing could mitigate the effects of changes in community composition on ANPP-precipitation relationships. Utilization of the same, shallow source of soil water would result in a degree of species redundancy, reducing the effect species losses in heavily-grazed communities. Furthermore, these results suggest that heavy grazing would reduce rain-use efficiency, at least in years with above-average rainfall, as water infiltrating to a deeper depth would not be utilized. In contrast, rain-use efficiency may be enhanced in dry years with low water availability at depth, as the majority of individuals would be relying on shallow soil water anyway. Heavy, non-selective grazing is therefore predicted to weaken the linear relationship between ANPP and precipitation.

While the results of this study have important implications for the effect of changing precipitation patterns on the ecosystem productivity, they are based on the short-term growth of individual plants. Long-term studies conducted at the ecosystem level are vital to validate such results. Furthermore the generality of such results needs to be tested in other ecosystems, particularly "full bucket" systems where substantial winter precipitation leads high water contents in deeper soil layers at the start of the growing season. Finally, the effects of other grazing regimes, particularly selective grazing, may alter the effect of compositional changes on ANPP-precipitation relationships in other ways.

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TABLES AND FIGURES

Table 4.1. Location and environmental variables for the five sites where the experiment was conducted. “MAP” = mean annual precipitation (parentheses indicate the average percentage that occurs during the growing season). “Rooting depth” refers to the approximate maximum grass root depth observed when installing soil probes. “Species richness” is the numbers of grass species (Poaceae) recorded within the study site (all were C_4).

Site	Location	Coordinates	MAP (mm)	Soils	Rooting depth (cm)	Summer temperatures (°C)	Burning history	Species richness
Basalts	Suikerbosrand Nature Reserve	26° 28.105’ S 28° 11.270’ E	665 (68%)[2]	Basalt derived. Clay-rich.	55	mean max: 25 mean min: 13 [3]	Burnt every 3 years; burnt immediately prior to start of experiment and prior to 3rd season	14
Nkuhlu	Kruger National Park, Nkuhlu large mammal exclosure	24° 59.333’ S 31° 46.458’ E	550 (87%)[4]	Granite derived. Sandy.	60	mean max: 33 mean min: 18 [5]	Previous burning irregular; no burning since 2 years prior to experiment	22
Sands	Suikerbosrand Nature Reserve	26° 31.607’ S 28° 17.065’ E	665 (68%)[2]	Sandstone derived. Sandy.	60	mean max: 25 mean min: 13 [3]	Burnt every 3 years; burnt prior to start of experiment and prior to 3rd season	29
Satara	Kruger National Park, exclosure within the Satara buffalo camp	24° 40.486’ S 31° 74.756’ E	544 (88%)[4]	Basalt derived Clay-rich.	75	mean max: 32 mean min: 19 [4]	Burnt approximately every 3 years; no burning since 1 year prior to experiment	12
Shabeni	Kruger National Park, exclosure on the Shabeni burn plots	25° 08.716’ S 31° 14.083’ E	737 (87%)[4]	Granite derived Sandy.	90	mean max: 31 mean min: 17 [4]	Previously burnt every 2 years. No burning since 1 year prior to experiment.	28

[1] Hayden (1998); [2] Reilly and Panagos (2002); [3] South African Weather service data Johannesburg , 1961-1990 (www.weathersa.co.za); [4] Nick Zambatis, Scientific Services, Kruger National Park, 1950 – 1990.

Table 4.2. Regression results for each of the species measured at each of the five sites, for the control and defoliation treatment for the control treatment, and the number of individuals used in the defoliation treatment. For the control treatment, model terms and r^2 of the models that best predicted growth rate are shown, and "n" is the number of sampling intervals for each regression. For the defoliation treatment, "Depth" refers to the water depth at which average soil water content produced the highest mean r^2 with growth rate, based on linear regressions for each individual measured, and "n" is the number of individuals used in the calculation of mean r^2. I = plant size (the average biomass of a species at the start of a sampling interval), A = average soil water content at 6.5cm, B = average soil water content measured at the base of the A horizon; C = average soil water content measured at the base of the rooting zone.

Site	Species	Control treatment			Defoliation treatment		
		n	Terms	r^2	n	Depth	Mean r^2
Basalts	Brachiaria serrata *(Thunb.) Stapf*	14	C	0.07	16	A	0.17 (0.04)
Basalts	Eragrostis chloromelas *Steud.*	12	C – C x I	0.65	16	A	0.17 (0.04)
Basalts	*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult.	14	A	0.21	15	A	0.25 (0.04)
Basalts	*Hyparrhenia hirta* (L.) Stapf	14	C	0.18	18	A	0.17 (0.04)
Nkuhlu	*Aristida congesta* subsp. *barbicollis* Roem. & Schult.	15	A	0.17	21	A	0.23 (0.06)
Nkuhlu	*Digitaria eriantha* Steud.	10	A	0.49	17	B	0.32 (0.06)
Nkuhlu	*Eragrostis superba* Peyr.	12	B	0.58	14	B	0.23 (0.06)
Nkuhlu	*Tragus berteronianus* Schult.	15	B – B x I	0.55	24	B	0.43 (0.07)
Sands	*Brachiaria serrata* (Thunb.) Stapf	15	C – I	0.77	16	A	0.26 (0.04)
Sands	*Eragrostis chloromelas*[1] Steud.	15	C – I	0.41	13	C	0.40 (0.06)
Sands	*Elionurus muticus* (Spreng.) Kuntze.	15	C – I	0.12	17	C	0.41 (0.06)
Sands	*Setaria sphacelata* var. *torta* (Stapf) Clayton	15	A – A x I	0.42	16	A	0.09 (0.04)
Satara	*Bothriocloa radicans* (Lehm.) A. Camus	13	B + B x I	0.83	20	B	0.45 (0.05)
Satara	*Digitaria eriantha* Steud.	13	B	0.10	18	B	0.54 (0.05)
Satara	*Themeda triandra* Forssk.	13	B + B x I	0.75	25	B	0.48 (0.06)
Satara	*Urochloa mossambicensis* (Hack.) Dandy	13	C – I	0.73	17	B	0.48 (0.06)
Shabeni	*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult.	18	A – I	0.34	18	A	0.38 (0.05)
Shabeni	*Hyperthelia dissoluta* (Steud.) Clayton	16	C – I	0.66	23	B	0.13 (0.05)
Shabeni	*Hyparrhenia filipendula* (Hochst.) Stapf	16	C – I	0.53	13	A	0.36 (0.06)
Shabeni	*Setaria sphacelata* var. *sphacelata* (Stapf) Clayton	16	B – I	0.39	15	A	0.15 (0.05)

[1] possibly hybridized with *Eragrostis curvula* Schrad. (Nees)

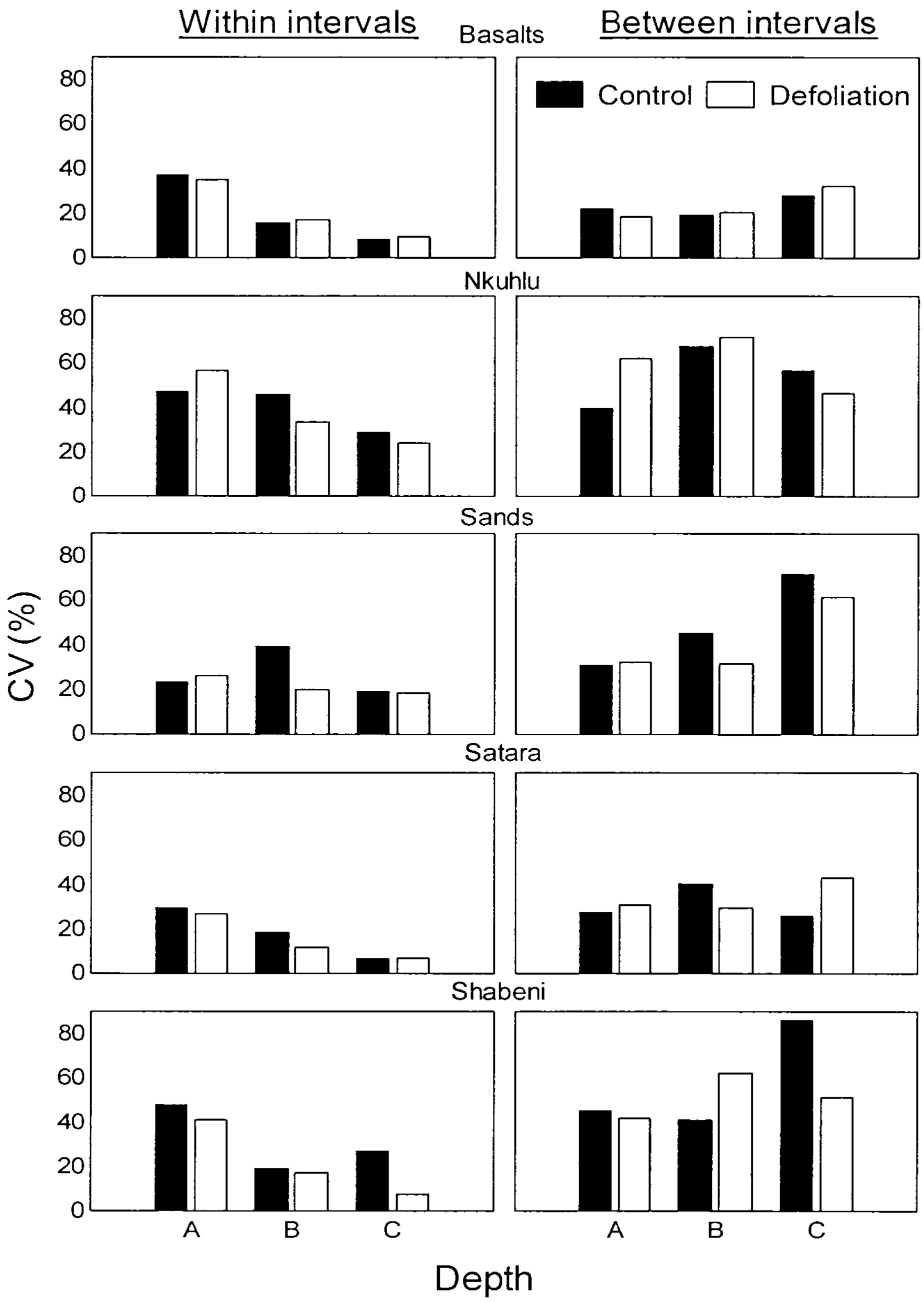

Figure 4.1. Variation in volumetric soil water content within and between sampling intervals for the control and defoliation treatment at each site. Within-interval variation is the mean of the CV for each interval (calculated from the daily means for each interval). Between-interval variation is the CV calculated from the overall mean VWC for each interval.

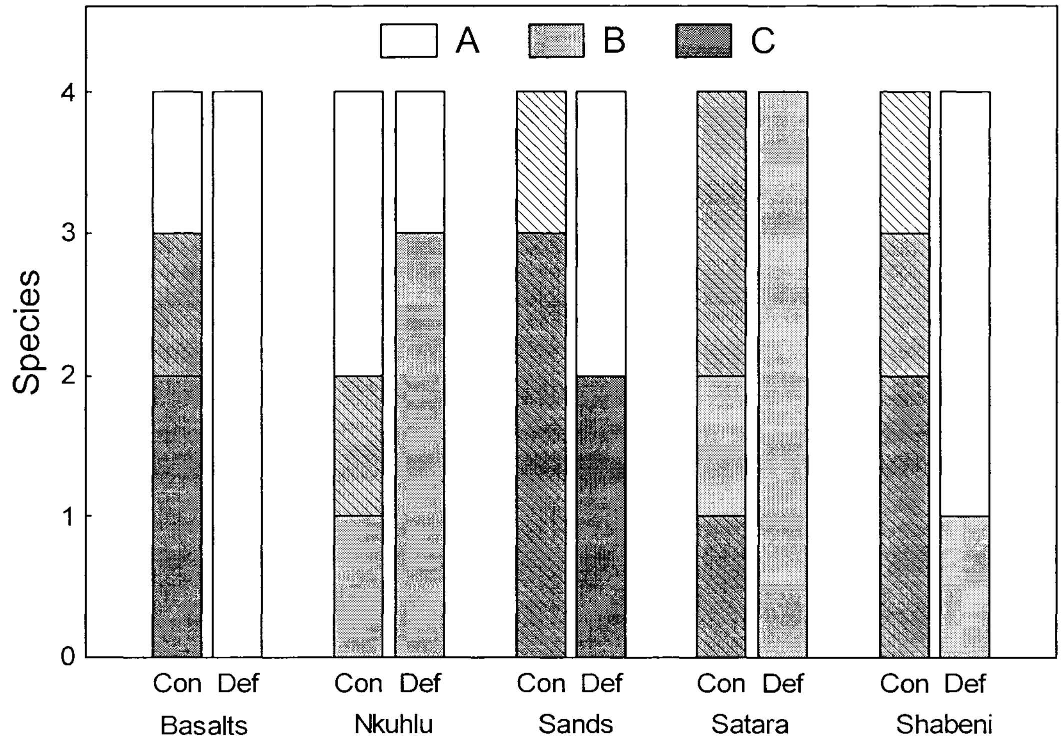

Figure 4.2. Best predictors of growth rates in the control (Con) and defoliation (Def) treatment. For the control treatment, bars show the numbers of species for which mean water content at a particular depth (A, B or C) best predicted growth rate. Hatching indicates an effect of plant size. For the defoliation treatment, bars show indicate which variable produced the highest mean r^2 of growth rate regressions for replicate individuals of each species.

CHAPTER 5

Functional groups and predictive traits for perennial C_4 grasses

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ABSTRACT

Precipitation is the primary determinant of the above-ground net primary productivity (ANPP) of grasslands, but changes in grass community composition can alter the effect of precipitation on productivity. Co-occurring species of grasses can differ substantially in terms of their above-ground growth responses to precipitation, indicating that changes in precipitation patterns may drive changes in community composition, and that changes in community composition may feed back into changes in ANPP at the ecosystem level. Furthermore, grazing can alter species-specific growth responses to precipitation. To determine whether grass species show similar growth responses to both precipitation and grazing, 20 C_4 perennial species from a range of grassland and savanna sites were grouped according to intra-seasonal growth responses to soil water content at four depths and repeated defoliation. No association was found between optimal depth of soil water utilization, changes in depth of soil water utilization with defoliation, and tolerance of defoliation. This indicates a high degree of functional diversity within C_4 grasses, and suggests that predicting the effects of changes in species composition on ANPP will be complex for many grass communities. Groups of easily-measured, above-ground traits were identified that could predict optimal depth of soil water utilization and changes in soil water utilization with defoliation, but not tolerance of defoliation. Further research is required to determine whether these predictive traits are functional.

INTRODUCTION

Individual species may play an important role in determining ecosystem responses to environmental changes, particularly dominant species (Smith and Knapp 2003). In grasslands and savannas, above-ground net primary productivity (ANPP) is determined primarily by precipitation (Smoliak 1986, Lauenroth & Sala 1992, Briggs & Knapp 1995, Jobbagy & Sala 2000, O'Connor *et al.* 2001, Chidumayo 2003, McNaughton *et al.* 2003, Knapp *et al.* 2006, Nippert *et al.* 2006), but grass community composition has been shown to alter both ANPP and the effect of precipitation on ANPP (Kelly & Walker 1976, O'Connor *et al.* 2001, Suttle *et al.* 2007, Swemmer *et al.* 2007). The effects of changes in community composition on productivity-precipitation relationships at the ecosystem scale will depend on the degree to which dominant and potentially dominant species differ in terms of their growth responses to precipitation. Recent research suggests that above-ground growth responses to precipitation can vary considerably amongst co-occurring grass species (Swemmer & Knapp 2007a,b).

Cognizance of variation amongst co-occurring species, in terms of their responses to environmental factors, has led to various attempts to classify species into functional groups, originally as means of understanding and community composition and dynamics (e.g. Grime 1979). More recently, attempts to predict changes in ecosystem function resulting from changes in community composition has led to classifications of species on the basis of their effects on ecosystem function (leading to a distinction between "response" and "effect" functional groups - Lavorel & Garnier 2002, Petchy & Gaston

2006). Species are usually assigned to functional groups on the basis of functional traits, i.e. traits that determine a species function (response or effect) and can be used to generalize functions to other species, or even communities (Lavorel *et al.* 2005). However, the functional significance of the traits used is often not clear, as phenotypic plasticity between habitats or evolutionary constraints can create the appearance of functionality in traits that are unimportant (Reich *et al.* 2003). This limits the value of functional traits for predicting whole-plant functions.

In this study we use direct measures of whole-plant growth to classify C_4 grasses according to their responses to two environmental factors that affect the relationship between precipitation and ANPP in grasslands and savannas: soil water availability and defoliation (a surrogate for grazing). We then use these functional groupings to address two exploratory questions. Firstly, is there any association between responses to soil water availability and responses to defoliation? If evolutionary trade-offs have restricted the range of adaptations for utilizing soil water (e.g. Meinzer 2003) and coping with defoliation, then certain combinations of "soil water" and "defoliation" functional groups may not occur. This would make predictions of the dynamics of C_4 grass communities simpler. Secondly, are there easily-measured traits that define the functional groups? Having established species' function directly, we test whether easily-measured traits ('soft traits' *sensu* Hodgson *et al.* 1999) can predict patterns of soil water utilization and defoliation tolerance. While such traits may not necessarily be functional, they would allow for generalization to other species, a vital step for extrapolating the established functions to other species in other environments.

METHODS

Experimental design

Data were collected from seven sites located in pristine, un-ploughed perennial grasslands or savannas dominated by C_4 perennial grasses. Two of the sites were located in the USA: one in a mesic grassland in eastern Kansas and one in a semi-arid grassland in central Colorado. The remaining sites were located in South Africa: three in savannas within the Kruger National Park and two in grasslands within the Suikerbosrand Nature Reserve. Table 5.1 summarizes the main environmental conditions at the sites. All of the sites are fenced and ungrazed, and with the exception of Konza, had a long history of grazing prior to the start of the experiment.

At each site, the four most common C_4 grass species were selected for sampling (species and site named are listed in the Appendix of Swemmer & Knapp, chapter 3). All except one were perennial. Six of the 28 species selected occurred at more than one site. Two pairs of sites shared two of these species, while another two pairs of sites each shared one species. These overlapping species were treated as separate species at each site. Individual tufts were clipped at regular intervals (17 to 50 days) during the growing season. Sampling intervals were deliberately shortened during wet periods when growth was more rapid in an attempt to measure a similar amount of growth in each interval. A defoliation and control treatment were established at each site with two plots per treatment. Each defoliation plot was located adjacent to a control plot. The proximity of each pair of defoliation and control plots varied from adjacent (Basalts and Sands) to approximately 100m (Konza). Defoliation treatment plot sizes varied from 3m x 3m (Basalts and Sands) to 5m x 5m (Konza). Control plots were larger, extending away from the adjacent clip plots as far as was required to locate sufficient numbers of individuals to sample. None of the plots contained large trees or were shaded by canopies of nearby trees. Seven or eight individuals per species were typically clipped per plot at each sampling interval.

In the defoliation treatment randomly selected tufts were marked at the start of the experiment and clipped to a height of 2 - 3cm at the end of each interval. Clipped biomass therefore consisted only of regrowth since the previous harvest. Individuals were marked and labeled using with a plastic-coated loop of wire placed around the base of the tuft. After the marked tufts were clipped at the end of an interval, all other grass tufts and forbs in the plots were clipped down to the same height and this biomass removed from the plot. If a marked individual died, a replacement was selected from the same plot, thus maintaining a constant number of replicates per plot (for some of the less common species, a shortage of additional individuals resulted in a reduction in replicates by the end of the experiment).

In the control treatment, individuals that had not been clipped previously were randomly selected at the end of each interval. These were then clipped down to the crown and marked with a flag to ensure they would not be clipped again. For strongly rhizomatous species, an individual was defined as a cluster of tillers that appeared to originate from the same rhizome. A new pair of control plots was established for each growing season and, in most cases, all plant material clipped to crown height prior to the onset of a season's growth, if the site had not been burnt. Thus all clipped material in the control treatment consisted of only current season growth. For one season at the Sands and Basalts sites, and for all three seasons at Nkuhlu, control plots were neither burnt nor clipped down prior to the start of the growing season. In these cases, into senesced material from the previous season was separated out on the basis of the color of senesced leaves.

Current season's biomass for each harvest, from each treatment, was dried to a constant mass (usually at 60°C for three days) and separated into leaf (leaf blades), green structural material (sheaths and green culms), culms (lignified, brown culms), inflorescences (live inflorescences, including peduncles) and senesced material (senesced material still attached to the plant, including necrotic leaf tips) before weighing.

Sampling was conducted for three growing seasons (between September 2003 and July 2006). Due to logistical constraints, the following periods could not be sampled: the second half of the first growing season at the Basalts and Sands sites, the control plots at Satara, and for three species in the control plots at Shabeni. Similarly, for the second half of the third growing season, sampling was not possible at Konza and Boulder. These represented no more than 15% of the possible measurements that could have been made over the three year study period.

Functional groups

Four variables relating to above-ground growth were analyzed. Two were categorical variables based on above-ground growth rates in the control treatment. One was a categorical variable relating to the effect of the defoliation treatment on water utilization. The fourth was a quantitative measure of the effect of defoliation on biomass production. As the first three variables were based on growth responses to changes in soil water content, only the 20 species from five sites where soil water content was measured were included in their respective functional groups.

For each sampling interval at each site, the average soil water content at each of three depths was calculated, based on hourly volumetric water content measured by buried capacitance probes (described in detail by Swemmer & Knapp 2007b). For each species, a growth rate for each interval was calculated as the increment in average biomass between harvests, divided by the length of the interval. Linear regressions were then used to test which water availability variable (interval rainfall or average soil water content at one of three depths) best predicted growth rate. The best predictor of growth rate was used here as an estimate of the **optimum depth** of water utilization for each species. Species were grouped into two optimum depth groups: *shallow*, for species with rainfall or depth A (6.5cm) as the best predictor; and *deep*, for species that had the strongest growth relationship with soil water content at a deeper depth (either the base of the A horizon or the maximum rooting depth). This provided two balanced groups of reasonable size (10 replicates each).

For many species, including the effect of plant size (the average above-ground biomass of individuals at the start of an interval) was found to improve the linear regressions of above-ground growth and rainfall or soil water (Swemmer & Knapp 2007a,b). The effect of plant size may have been the result of inherent limitations to growth rates as individuals grew larger (such as self-shading), but could also have resulted from other factors limiting growth as the growing season progressed, such phenology (some species appeared to stop growing once they had flowered), competition (e.g. greater shading by neighbors) or declining soil nutrient availability. This effect of plant size is therefore referred to here as **seasonal constraints**, and species were categorized into two groups (*affected* or *not affected*).

The optimum depth of soil water utilization for the marked individuals in the defoliation treatment was calculated in a similar manner as in the control treatment (Swemmer & Knapp 2007b). This was found to change for many species. Species were therefore assigned to two **depth change** groups: *same,* for species with the same optimum depth in both treatments, or a deeper optimum depth in the defoliation treatment; and *shallower,* for species with a shallower optimum depth in the defoliation treatment. Species with interval rainfall as the best predictor in both treatments were excluded (this reduced the sample size from 20 to 17).

Finally, the effect of regular defoliation on growth rates was determined quantitatively. **Defoliation tolerance** was calculated for each species as the percentage of sampling intervals for which average biomass was higher in the defoliation treatment than in the control treatment.

Traits

Twelve traits where measured during the course of the experiment, on individuals in both treatments. In the control treatment, measurements were made on individuals that had not been clipped previously.

Maximum size

Maximum size was calculated for the control treatment only, as the maximum value of live biomass for all individuals harvested for each species. Maximum growth rate, the maximum rate from all intervals, was highly correlated with maximum size ($r = 0.90$, $p < 0.01$) and was not therefore used in any analyses.

Biomass allocation

Biomass allocation variables were calculated using data from two full growing seasons (data from half-seasons were excluded). A separate variable was calculated for each live component of harvested material (leaf, green structural material, culm, and inflorescence) as a mass fractions of total live biomass, e.g. leaf mass fraction = leaf / total live. The allocation of senesced material was calculated as a fraction of total biomass: senesced material / (live + senesced material). Each variable was only calculated for individuals that had biomass for the relevant variable, e.g. senesced mass fraction was calculated only for individuals for which some senesced material was present. Each allocation variable is therefore a measure of the average allocation to that variable for plants at a stage when allocation to the variable occurred.

For the control treatment, data for each allocation variable were combined across all intervals for each species. For the defoliation treatment, data were combined for each individual to account for the repeated measures design in this treatment. Distributions of these combined data were often skewed to the right. The median was therefore used as a measure of a species' average value for each trait, except for average leaf mass fraction in the defoliation treatment. For the latter the mean was used, as the median equaled one for certain species (a result of many harvests in the defoliation treatment consisting entirely of leaf blades). Most of the average allocation variables calculated in this way were correlated. Principle components analysis was therefore used to reduce these variables to components that better described variation in allocation patterns. A separate analysis was conducted for each treatment. In addition separate analyses were conducted for the 20 species used in the categorical functional groups (optimal depth, seasonal constraints and depth change), and for all 28 species, from all seven sites, used in the analysis of defoliation tolerance. The results of the PCAs are shown in Table 5.2. In all analyses, the first component (PC 1) was strongly correlated with allocation to leaves and negatively with allocation to structural materials. For the subset of 20 species, the second component (PC 2) was correlated with allocation to inflorescences and senesced material, and the third component (PC 3) positively correlated with allocation to inflorescences and negatively with allocation to brown structural material. For all 28 species, PC 2 for the control treatment was strongly correlated with allocation to inflorescences, and PC 3 with allocation to senesced material. In the defoliation treatment, PC 2 was highly correlated with allocation to inflorescences for both data sets.

Tiller numbers

The number of tillers per individual was counted at each harvest during the first growing season. Typically 10 individuals per species were counted in each treatment. Maximum tiller number was calculated simply as the maximum number of tillers recorded for each species in each treatment. As a measure of average tiller number, the number of tillers in the middle of the first growing season (usually sampling interval three) was used. As the distribution of tiller numbers within a treatment was usually skewed right, the geometric mean was used as the measure of average tiller number.

Leaf carbon and nitrogen

Total carbon (C) and nitrogen (N) concentration was measured on immature green leaf blades from 4 – 6 harvests, depending on the site. Harvests were specifically chosen to include at least two intervals from early in growing season and with high rainfall (when growth was rapid for all species) and at least two intervals from intervals later in the growing season and with lower rainfall. Equal numbers of oven-dried blades ($n = 1 - 8$) from each individual harvested in each plot were combined for analyses. Only young blades, not fully expanded with no necrosis, were selected. Samples were ground through a 0.5mm mesh with a Cyclone Mill (UDY Corporation, Fort Collins, Colorado) and analyzed for total C and N using a Carlo-Erba CNH Analyser.

ANOVA conducted for each site revealed no significant differences in leaf N between each plot in the control treatment. However for all the sites except Satara, samples from the early season intervals had significantly higher leaf N than those from later in the season. Therefore, for each species in the control treatment, data were first averaged by time of the season and the mean of these values used as a measure of average leaf N. In the defoliation treatment, differences between plots and differences between time of season were never significant, and average leaf N was simply calculated as the mean of all samples per species. Leaf C/N was calculated for each sample and average leaf C/N calculated in the same way as for leaf N.

Specific leaf area

Specific leaf area (SLA) was measured once at each site, in the first or second interval of the third growing season, when all leaves were green. 2 - 4 individuals per species were sampled in each plot. Samples were taken by punching circular disks at regular spacings along the length of a leaf blade using a belt punch (2 to 10 disks per blade depending on blade length). For most samples a punch diameter of 2.5mm was used. For thin leaves a diameter of 2mm was used, while for particularly wide leaves, a paper punch was used (diameter = 6mm). For blades that were thinner than the smallest punch hole, rectangular sections were cut and their length and width measured at the time of cutting to calculate area. For all species, samples were collected from the first fully expanded blade (usually leaf number two), and usually one blade sampled per individual. Where there were limited numbers of individuals of a species in a plot, additional blades were sampled from some individuals so that an approximately equal number of blades were sampled for each species in each treatment.

Plasticity traits

Changes in traits resulting from regular defoliation were calculated for leaf N, SLA, average tiller number and leaf mass fraction. Differences were calculated as the percentage change in with defoliation treatment for each interval as:

(defoliation value – control value) / control value * 100%.

For leaf mass fraction, intervals medians were calculated for all individuals harvested in each treatment. The mean percentage difference for all intervals was used as a measure of plasticity for each of these traits, for each species.

Relating traits to functions

For the categorical functions, discriminant function analysis (DFA) was used to identify groups of traits that predicted which functional group a species belonged to. Only traits for the control treatment were used in the optimum depth and seasonal constraints analyses. Control treatment and plasticity traits were used for the depth change analysis. For each functional variable, all relevant traits were entered into a DFA model and the best subset selected on the basis of the misclassification rate (i.e. the percentage of cases misclassified by a model). None of the best models contained significantly correlated traits. For unbalanced groups, prior probabilities were set as equal (i.e. it was assumed that differences in sample sizes resulted from unequal sampling). Ln transformation was used to homogenize variances between groups where necessary. Residual plots were used to check for multivariate normality of errors, and outliers assessed on the basis of DFFITS values. Correlations and linear multiple regression were used to evaluate relationships between defoliation tolerance and all traits (control, defoliation and plasticity). All statistical analyses were performed using Statistica v6.1 (Statsoft Inc., Tulsa).

RESULTS

Functional groups

Two-way frequency tables indicated little association between the optimum depth and seasonal constraints groups (Table 5.3a). For species with a deep optimum rooting depth more species were affected by seasonal constraints than not, but this difference was not greater than what would be expected by chance (Pearson's $\chi^2 = 2.0$, $p = 0.16$). No association of between optimum depth and depth change with defoliation was evident either, other than the necessary absence of species with a shallow optimum depth and a change to a shallower depth (Table 5.3b). For the 10 species with a deep optimum depth, approximately equal numbers maintained a deep optimum depth as changed to shallower optimum depth. There was significant association between seasonal constraints and depth change (Pearson's $\chi^2 = 5.1$, $p = 0.02$; Table 5.3c). All the species not affected by seasonal constraints in the control treatment did not show a change to a shallower optimum depth in the defoliation treatment. Of the species that did show seasonal constraints, approximately equal proportions maintained the same optimum depth or had a shallower optimum depth. Mean defoliation tolerance did not differ significantly between categories in any of the three functional groups (Fig 5.1) and was not affected by the interaction of any pair of groups.

Predictive traits

Optimum depth group

The best DFA model for optimum soil depth group retained five traits (Table 5.4) and correctly classified 19 out of the 20 species. Species in the shallow category had (on average) smaller maximum sizes, lower leaf N, more tillers, higher PC 3 loadings (indicating greater allocation to inflorescences but less to culms) and lower PC 1 loadings (indicating greater allocation to leaves, but less to sheaths and culms). Leaf C/N and SLA were correlated with leaf N (leaf C/N: $r = -0.93$, $p < 0.01$; SLA: $r = 0.48$, $p = 0.03$) but neither produced a better model when used in place of leaf N. One species (*Eragrostis chloromelas* at the Basalts site) was an outlier - excluding it did not change the misclassification rate but did result in the dropping of PC 3, and made the multivariate tests for leaf N and tiller number highly significant. Three of the five sites used in the analysis had species in both optimum depth categories. Only for maximum size, the strongest term in the model, were differences between the optimum depth groups consistent for each of these three sites (Fig 5.2). This was not the case for the three tribes with species in each category, although leaf N did show a consistent trend within these tribes (Fig 5.2).

Seasonal constraints group

The best DFA model for the seasonal constraints group correctly classified 18 out of 20 species using three traits (Table 5.4). Species affected by seasonal constraints had higher SLA, fewer tillers and lower PC 1 loadings (high allocation to leaves and low allocation to sheaths and culms). Within the three sites having species in both categories, consistent differences were evident for tiller numbers (Fig 5.3), but not for SLA and PC 1. None of the trends held within the three tribes (Androgoneae, Paniceae, Eragrosteae) having species in each category.

Depth change group

The best DFA model for discriminating between species according to changes in optimum rooting depth correctly classified all 17 species in the group. This model retained five traits with each contributing substantially to differences between the groups (Table 5.4). Species that maintained optimum depth had significantly smaller maximum sizes, lower SLA, more tillers, less increase in tiller numbers with defoliation, and higher scores for PC 3 (indicating greater allocation to inflorescences and less to culms and senesced material). One species (*Hyperthelia dissoluta*) was an outlier – excluding it resulted in the elimination of the weakest term, maximum size. Only two sites had more than one species in each category, and only the differences in tiller number were consistent for both sites (Fig 5.4). This was also the case for the two tribes having species in each category (Fig 5.4).

Defoliation tolerance

Correlations between traits and defoliation tolerance were weak, and most not significant at the 10% significance level (Table 5.5). Most of the traits with significant correlations were also correlated with each other. The stronger correlations ($p < 0.05$) indicated that species with greater tolerance had higher leaf N, lower leaf C/N and higher SLA in both treatments, with leaf N in the defoliation treatment being the strongest predictor. When uncorrelated traits where combined in multiple regressions, the best model explained 44% of the variance in defoliation tolerance. This model contained leaf N for the defoliation treatment and PC 2 loadings for the control treatment (the latter indicating that species with greater tolerance had greater allocation to inflorescences and less to culms when not regularly defoliated). No consistent correlations were found for any of these traits for the four species at each of the seven sites. Correlations also differed between the two tribes containing the majority of the species. The overall effect of increasing tolerance with higher leaf N was evident for species in the Paniceae but not in the Andropogoneae (Fig 5.5). The same was true for the correlated traits of C/N, SLA and PC 1 loadings (defoliation treatment). ANCOVA indicated that this difference was statistically significant for leaf N measured in both the defoliation treatment (overall model: $F_{3,17} = 9.9$, $p < 0.01$; interaction of tribe x leaf N: $F_{1,17} = 7.7$, $p = 0.01$) and the control treatment (overall model: $F_{3,17} = 8.5$, $p < 0.01$; interaction of tribe x leaf N: $F_{1,17} = 10.2$, $p < 0.01$).

DISCUSSION

Functional groups

Grouping species according to their growth responses to soil water at different depths and regular defoliation revealed no meaningful association between these two response functions. This suggests that perennial C_4 grass species have evolved to fill a variety of niches relating to soil water utilization and tolerance of grazing. This reflects the occurrence of aridity and grazing throughout the evolution of the Poaceae (Stebbins 1981, Coughenour 1985). The only clear association between functional groups was that species not affected by seasonal constraints never showed a change to utilizing soil water at a shallower depth when regularly defoliated. This relationship is unlikely to be a result of physiological constraints, and likely indicates simply a lack of selective pressure for reducing allocation to roots following defoliation. A change to a shallower rooting depth is unlikely to confer greater tolerance of defoliation as soil water content was most variable at the shallowest depth (Swemmer & Knapp 2007b), and on average lower than at deeper depths (data not shown). Of the species that were affected by seasonal constraints, the change to a shallower depth for some probably indicates a trade-off of reducing allocation of assimilates below-ground in order to maintain above-ground growth under repeated defoliation (Coughenour and McNaughton 1984).

The lack of differences in defoliation tolerance between the optimum depth categories and the seasonal constraints categories was surprising. Species with deep optimum depths were expected to show poor regrowth following defoliation if they maintained a deep optimum depth, as this would require maintaining deep roots. However four out of ten species managed to continue utilizing deeper soil water when regularly defoliated, and did not show low defoliation tolerance. These species may have increased rates of photosynthesis of residual (unclipped) leaves to maintain allocation below-ground (Wallace *et al.* 1984), or may have been able to modify their root architecture to maintain the same surface area of roots at depth using less root biomass. Species not affected by seasonal constraints were also expected to show lower defoliation tolerance, as competition for light, in the absence of grazing, is likely to involve trade-offs that reduce defoliation tolerance (Huisman *et al.* 1999), and those species not affected by seasonal constraints were presumably good competitors for light. It is possible that such species have evolved highly plastic above-ground morphologies to cope with both light competition and defoliation. Alternatively, trade-offs associated with vulnerability to seasonal constraints may not necessarily involve greater defoliation tolerance. For example traits that deter grazers, rather than tolerate defoliation (such as high lignin content or low nutrient concentrations) may reduce competitive ability or otherwise constrain growth later in the growing season.

Predictive traits

Various suites of traits were identified that could accurately predict species occurrences in functional groups for three of the four functional variables. Individually these traits did not differ substantially between groups, and all overlapped. However the effect of the traits in combination - the multivariate effects revealed by the DFA – was significant.

The general lack of consistent differences within sites, for any one of these predictive traits, does not indicate a lack of generality of the multivariate results, as it is combined differences in the traits that have predictive value. These traits could be used to predict whether a species is likely to increase or decrease in abundance with a change in precipitation regime that affects water availability at a certain depth, or a change in grazing regime that affects the frequency of defoliation. Similarly, changes in ecosystem function (particularly ANPP) could be predicted from known changes in community composition, for a given precipitation and grazing regime. However, while these results were obtained for a wide range of species, in a wide range of habitats, extrapolation to other C_4 grasses should be done with caution, as a causal role in determining each function was not established for any of the traits. Differences in site-specific factors or phylogenetic constraints may reduce the association of these predictive traits and their respective functions, for different species in different environments. Measurements of these traits on other species that show similar changes in growth responses to soil water availability or defoliation would provide a test of how robust these predictions are. Applying the predictions to C_3 grasses would be more ambitious, but would provide a test of whether these results are relevant to grasses in general, or may provide insights into differences in soil water utilization and defoliation tolerance between these two major functional grass groups.

In contrast to the three categorically defined functions, the function of defoliation tolerance was not well predicted by any trait, or any combination of traits. The strongest predictor, leaf N, suggests that C_4 grasses require a high supply of N to regrow tissues lost to defoliation. This is supported by numerous studies showing that increased N availability increases regrowth following clipping, for a range of graminoid species (Ruess *et al.* 1983, Coughenour *et al.* 1985, Wallace *et al.* 1985, Georgiadis *et al.* 1989, Hamilton *et al.* 1998). Greater leaf N is associated with higher chlorophyll concentrates and higher rates of net photosynthesis (Lambers *et al.* 1998), which would enable faster regrowth following defoliation. Greater defoliation tolerance was also associated with higher SLA, but the correlation of leaf N and SLA makes it difficult to determine the relative importance of each. Other studies provide some evidence of an association between SLA and grazing tolerance: Caldwell *et al.* (1981), in a comparison of two C_3 grass species, found greater SLA for the one producing more regrowth, while Díaz *et al.* (2001) found higher SLA for herbaceous species abundant in heavily-grazed communities across a broad range of sites spanning two continents. However, Vesk *et al.* (2004) found no consistent change in SLA between grass species that either increase or decrease in abundance with heavy grazing, for range of arid and semi-arid rangeland sites. Correlation of leaf N and SLA has been found for a very large range of plants (Wright *et al.* 2004), and is part of a syndrome of traits separating faster-growing species with high rates of tissue turnover from slower growing species (Reich *et al.* 2003). The correlation between leaf N and SLA, and defoliation tolerance therefore suggests that the more tolerant species, in this study, belong to a broader group of faster-growing species.

The lack of a strong effect of the other traits on defoliation tolerance was surprising. Short plant height (Sternberg *et al.* 2000; Díaz *et al.* 2001) and a prostrate habit (Landsberg *et al.* 1999) have been associated with heavy grazing intensity for herbaceous plants in general. For grasses in a particular, a short-stature has been predicted to increase defoliation tolerance as shorter grasses maintain meristems below the reach of grazers, allowing enhanced tillering following defoliation (Coughenour *et al.* 1984). In this study, only a very weak association was found, in that allocation to culms (indicated by PC 2 values in the control treatment) was negatively correlated with defoliation tolerance. This supports other studies from rangeland and grassland sites that in which no association of plant height and grazing intensity was found (Vesk *et al.* 2004; De Bello *et al.* 2005), and indicates that short-stature is not a prerequisite for defoliation tolerance. The ability to increase tillers numbers following defoliation has also been predicted to improve regrowth following defoliation (Coughenour *et al.* 1984). However, while some studies support this (Booysen 1981, Richards *et al.* 1988, Simoes & Baruch 1991) others show no effect of changes in tiller number on regrowth (Hodgkinson *et al.* 1989, Klink 1994). In this study, both increases and decreases in tiller numbers were observed, but the percentage changes in tiller number was not correlated with defoliation tolerance, suggesting that plasticity in tiller production is also not a requirement for defoliation tolerance.

These results suggests that differences in defoliation tolerance cannot be accurately predicted from above-ground morphological traits for grasses in general. Rather it is likely that a combination of both morphological and physiological traits (such as nitrogen use efficiency, storage of assimilates below-ground and high rates of net photosynthesis - Caldwell and Richards 1981, Wallace and McNaughton 1984) are required to accurately predict grazing tolerance.

Functional traits

While the traits highlighted here may be accurate predictors of whole plant functions, no inferences can be made regarding causation. An association between a trait and a function could indicate an underlying cause (i.e. the trait is functional) or could simply result from covariance of the trait with one or more other (unmeasured) traits that are truly functional. The role of phylogenetic constraints creating covariance of unimportant traits with those that are adaptive has long been known (Harvey *et al.* 1995). Furthermore trait and site effects could be confounded, i.e. an association between a trait and a function could result from some underlying site-specific factor that is the cause of both (Adler *et al.* 2004). Comparing the relationship between a trait and a whole-plant function for different sites and different phylogenies provides a means to test for functionality. The greater the number of times a relationship occurs at different sites, or phylogenies, the greater the probability that the trait is the cause of the function, and not a result of covariance with other traits or coincidental site-specific factors. The design of this experiment did not allow for such tests, as there were not replicate species for every combination of site, phylogeny and functional group. However, comparing trends within sites and phylogenies (represented by tribes) did provide some insight into the functionality (or lack thereof) for the predictive traits identified.

These comparisons indicated that maximum size may be a functional trait, as it was consistently greater for species with a deep optimum depth for three sites and for two out of three tribes. A causal link between size and optimum depth is plausible, as a deep rooting depth would require a minimum amount of root biomass and allometric constraints probably restrict the amount of root biomass that a smaller plant can produce. Tiller number may also be functional, as it was generally higher for those species not affected by seasonal constraints. Again functionality is likely, as species that producer fewer, larger tillers are generally taller and less likely to have their growth constrained by shading later in the growing season. While tiller number was also consistently higher for species that were able to maintain their optimum rooting depth with regular defoliation, at least within two sites and two tribes, a putative cause for this is not obvious.

The relationship between leaf N and defoliation tolerance did not occur within many sites, indicating that effect of leaf N on defoliation tolerance was driven by a site factor, presumably different rates of soil N mineralization, and not by species differences. Indeed the mean defoliation tolerance and mean leaf N for each site were highly correlated ($r = 0.82$, $p = 0.03$). However, site and phylogeny were confounded to a certain extent, and the comparison of leaf N correlations between two tribes indicated that species in the Paniceae were able to utilize high availability of N to better tolerate defoliation, while those in the Andropogoneae were not. Therefore, while the association between leaf N and grazing tolerance was clearly not causal, it cannot be said whether species had greater tolerance of defoliation because of greater N availability where they were growing, or because they were able to utilize available N as a result of adaptive traits associated with their phylogeny.

Conclusion

Direct measurements of the above-ground growth responses of a range of C_4 grasses provided an opportunity to group species according to their responses to soil water availability and defoliation. This provided insights into how changes precipitation and grazing regimes may alter grass community composition. The lack of association between responses to these two major environmental factors suggests significant evolutionary divergence of key functions within the C_4 grasses, even amongst co-occurring species. While differences in responses to soil water availability could be predicted from groups of easily-measured traits, further research is required to establish whether these traits are functional, as well as which traits determine differences in defoliation tolerance.

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TABLES AND FIGURES

Table 5.1. Locations and environmental variables for the seven sites where the experiment was conducted. "MAP" = mean annual precipitation (parentheses indicate the approximate percentage of precipitation that occurs during the growing season). "Rooting depth" refers to the approximate maximum grass root depth observed when installing soil probes. "Species richness" is the numbers of species (Poaceae) recorded within the study site (parentheses shows the number of these that are C_3).

Site	Location	Coordinates	MAP (mm)	Soils	Rooting depth (cm)	Summer temperatures (°C)	Burning history	Species richness
Basalts	Suikerbosrand Nature Reserve	26° 28.105' S 28° 11.270' E	665 (68%)[2]	Basalt derived. Clay-rich. Ferrocrete present.	55	mean max: 25 mean min: 13 [4]	Burnt every 3 years; burnt immediately prior to start of experiment and prior to 3rd season	14 (0)
Boulder	City of Boulder Open Space System, Van Vleet property	39° 53.583' N 105° 14.700' W	457 (55%)[3]	Sandstone derived glacial outwash. Clay loam, shallow, rocky. [3]	-	mean max: 23 mean min: 12 [3]	No previous burning but burnt prior to 3rd season of experiment.	13 (8)
Konza	Konza Biological Research Station, watershed 1B	39° 4.308' N 96° 35.999' W	835 (75%)[1]	Limestone and shale derived. Clay rich, deep. [6]	-	mean max: 27 [7] mean min: 16	Annually in spring.	7 (1)
Nkuhlu	Kruger National Park, Nkuhlu large mammal exclosure	24° 59.333' S 31° 46.458' E	550 (87%)[5]	Granite derived. Sandy.	60	mean max: 33 mean min: 18 [5]	Previous burning irregular; no burning since 2 years prior to experiment	22 (0)
Sands	Suikerbosrand Nature Reserve	26° 31.607' S 28° 17.065' E	665 (68%)[2]	Sandstone derived. Sandy.	60	mean max: 25 mean min: 13 [4]	Burnt every 3 years; burnt prior to start of experiment and prior to 3rd season	29 (0)
Satara	Kruger National Park, exclosure within the Satara buffalo camp	24° 40.486' S 31° 74.756' E	544 (88%)[5]	Basalt derived Clay-rich. Calcrete present	75	mean max: 32 mean min: 19 [5]	Burnt approximately every 3 years; no burning since 1 year prior to experiment	12 (0)
Shabeni	Kruger National Park, Exclosure on the Shabeni burn plots	25° 08.716' S 31° 14.083' E	737 (87%)[5]	Granite derived Sandy.	90	mean max: 31 mean min: 17 [5]	Previously burnt every 2 years. No burning since 1 year prior to experiment.	28 (0)

[1] Hayden (1998); [2] Reilly and Panagos (2002); [3] City of Boulder Open Space and Mountain Parks (1986); [4] South African Weather service data Johannesburg , 1961-1990 (www.weathersa.co.za); ; [5] Nick Zambatis, Scientific Services, Kruger National Park, 1950 - 1990; [6] Ransom et al. (1998); [7] Fay et al. (2003).

Table 5.2. PCA of the allocation variables for the control and defoliation treatment for a) the 20 species from the five sites where soil water content was measured, and b) all 28 species in the experiment. Factor loadings (correlations) are given for each PC used in the trait analyses. Each allocation variable refers to a mass fraction.

	Control treatment			**Defoliation treatment**	
	PC 1	PC 2	PC 3	PC 1	PC 2
	a) Subset of 20 species				
Variance explained (%)	47	25	20	57	22
Leaf	-0.99	-0.07	-0.02	-0.91	0.08
Green structural	0.88	-0.13	0.25	0.91	-0.26
Brown structural	0.74	0.10	-0.60	0.80	0.32
Inflorescence	0.17	0.74	0.61	0.36	0.88
Senesced	-0.20	0.82	-0.44	0.67	0.38
	b) All 28 species				
Variance explained (%)	47	23	22	56	20
Leaf	0.98	0.10	0.06	0.90	0.17
Green structural	-0.90	-0.09	0.15	-0.90	-0.30
Brown structural	-0.66	0.43	-0.53	-0.68	-0.38
Inflorescence	-0.16	-0.96	-0.10	-0.54	0.74
Senesced	0.33	-0.16	-0.89	-0.66	0.74

Table 5.3. Frequency of the subset of 20 species in the paired combinations of functional groups. a) optimum depth and seasonal constraints, b) optimum depth and depth change and c) seasonal constraints and depth change

a)	Optimum depth		
Seasonal constraints	Shallow	Deep	**Total**
Not affected	5	2	**7**
Affected	5	8	**13**
Total	**10**	**10**	**20**

b)	Optimum depth		
Depth change	Shallow	Deep	**Total**
Same	7	4	**11**
Shallower	0	6	**6**
Total	7	**10**	**17**

c)	Depth change		
Seasonal constraints	Same	Shallower	**Total**
Not affected	6	0	**6**
Affected	5	6	**11**
Total	**11**	**6**	**17**

Table 5.4. Best discriminant function analysis models selected for the optimum depth, seasonal constraints and depth change functional groups. Means (SD) trait values are shown for each category in each group. The Wilks lambda statistic indicates the contribution of each trait to the discriminatory power of the model (smaller values indicate a larger contribution). p values are for multivariate F tests. Maximum size values are back transformed from ln transformed values used in the model. "Tillers" is to the average number of tiller per individual. "Δ tillers" is the percentage increase in average tiller number in the defoliation treatment. PC 1, 2 and 3 are principle component factor loadings for five biomass allocation variables for the control treatment (see Table 2).

Trait			**Wilks lambda**	**p**
	Optimum depth			
	Shallow	Deep		
Max. size	21.0 (2.20)	50.0 (1.89)	0.63	0.01
Leaf N (%)	1.93 (0.40)	2.13 (0.43)	0.81	0.09
Tillers	19 (7)	16 (9)	0.87	0.17
PC 3	0.32 (0.91)	-0.32 (1.00)	0.97	0.56
PC 1	-0.44 (1.63)	0.44 (1.38)	0.99	0.67
	Seasonal constraints			
	Not affected	Affected		
PC 1	0.44 (1.5)	-0.24 (1.6)	0.59	<0.01
SLA (m^2 kg^{-1})	26.1 (3.48)	31.8 (10.4)	0.64	<0.01
Tillers	22 (10)	15 (6.5)	0.65	<0.01
	Depth change			
	Same	Shallower		
Tillers	20 (8)	12 (6)	0.41	< 0.01
Δ tillers (%)	7 (24)	34 (46)	0.42	< 0.01
PC 3	0.28 (0.96)	-0.39 (1.16)	0.44	< 0.01
SLA (m^2 kg^{-1})	30.3 (8.7)	30.5 (5.9)	0.62	0.025
Max. size	27.4 (2.7)	43.6 (2.0)	0.69	0.047

Table 5.5. Correlation coefficients for all traits correlated with defoliation tolerance ($p < 0.1$) for the 28 species from all seven sites. Bold coefficients show significance at $p = 0.05$. Parentheses indicate distinguish traits measured in the control (C) and defoliation treatment (D).

	Defoliation tolerance	**Leaf N (C)**	**Leaf C/N (C)**	**SLA (C)**	**Leaf N (D)**	**Leaf C/N (D)**	**SLA (D)**	**PC 2 (C)**	**PC 1 (D)**
Leaf N (C)	**0.47**								
Leaf C/N (C)	**-0.44**	**-0.94**							
SLA (C)	**0.40**	**0.66**	**-0.64**						
Leaf N (D)	**0.61**	**0.93**	**-0.84**	**0.66**					
Leaf C/N (D)	**-0.61**	**-0.94**	**0.93**	**-0.67**	**-0.95**				
SLA (D)	**0.42**	**0.66**	**-0.63**	**0.95**	**0.65**	**-0.65**			
PC 2 (C)	-0.34	-0.21	0.26	-0.06	-0.12	0.20	-0.07		
PC 1 (D)	-0.36	**-0.68**	**0.64**	**-0.67**	**-0.65**	**0.62**	**-0.66**	0.35	
PC 2 (D)	0.34	0.22	-0.22	-0.02	0.23	-0.27	0.02	-0.52	-0.01

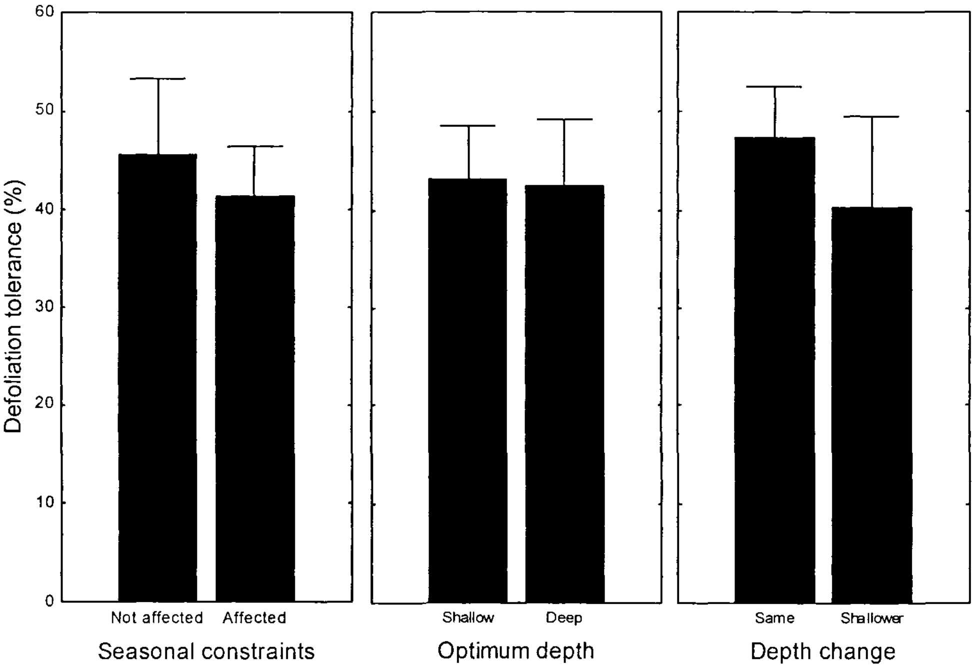

Figure 5.1. Mean defoliation tolerance for the species in each group of the optimum depth, seasonal constraints, and depth change variables. Mean defoliation is the percentage of sampling intervals with greater biomass produced in the defoliation treatment than the control treatment. Error bars show 1 SE. Refer to Table 3 for sample sizes in each category.

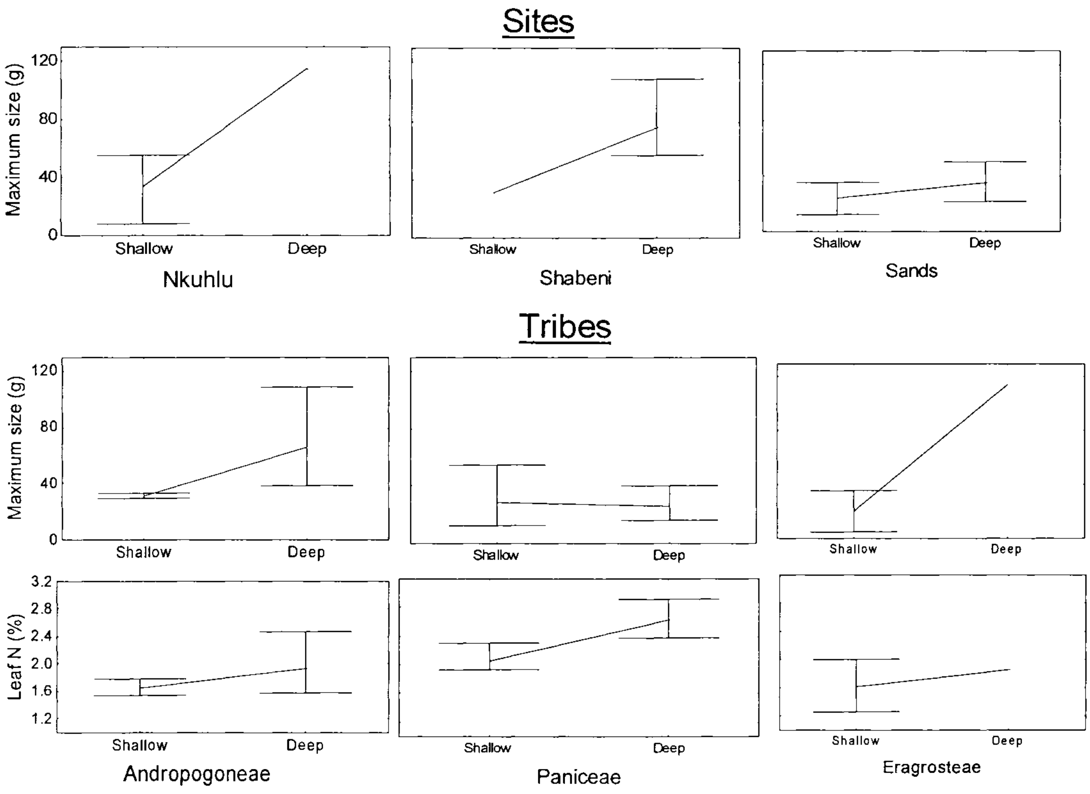

Figure 5.2. Means and ranges for the two traits (maximum size and leaf nitrogen) that showed consistent differences between sites and tribes for the two optimal depth groups. Only sites and tribes with species in each group are shown.

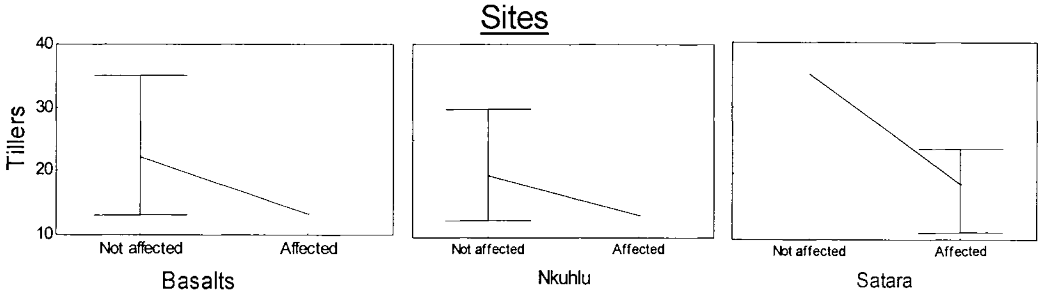

Figure 5.3. Means and ranges for the average number of tillers per individual in the two seasonal constraints groups, for the three sites with species in each group.

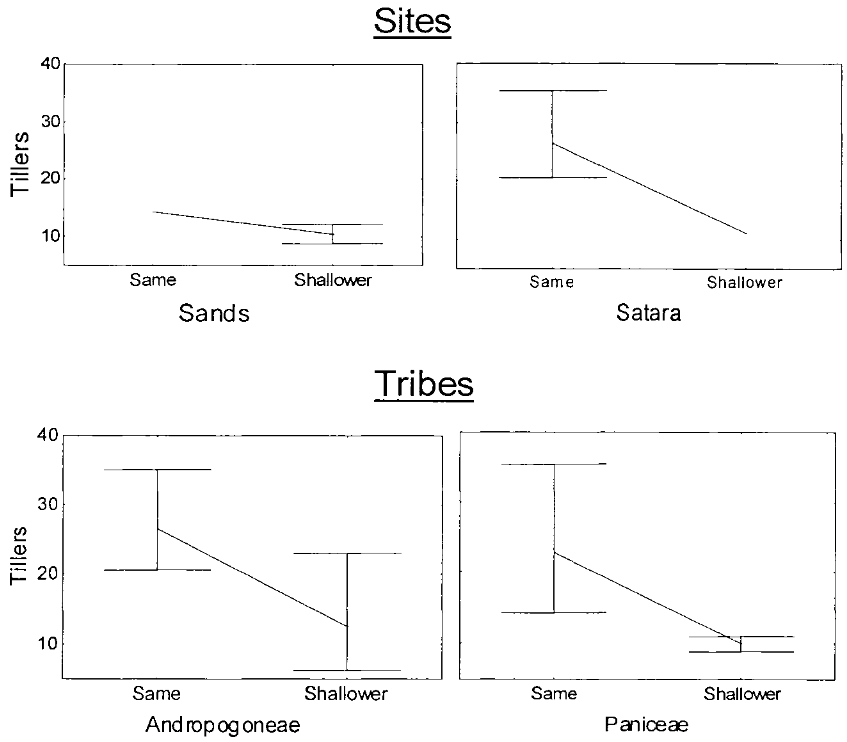

Figure 5.4. Means and ranges for average number of tillers per individual in the two depth change groups, for the two sites and tribes with species in each group.

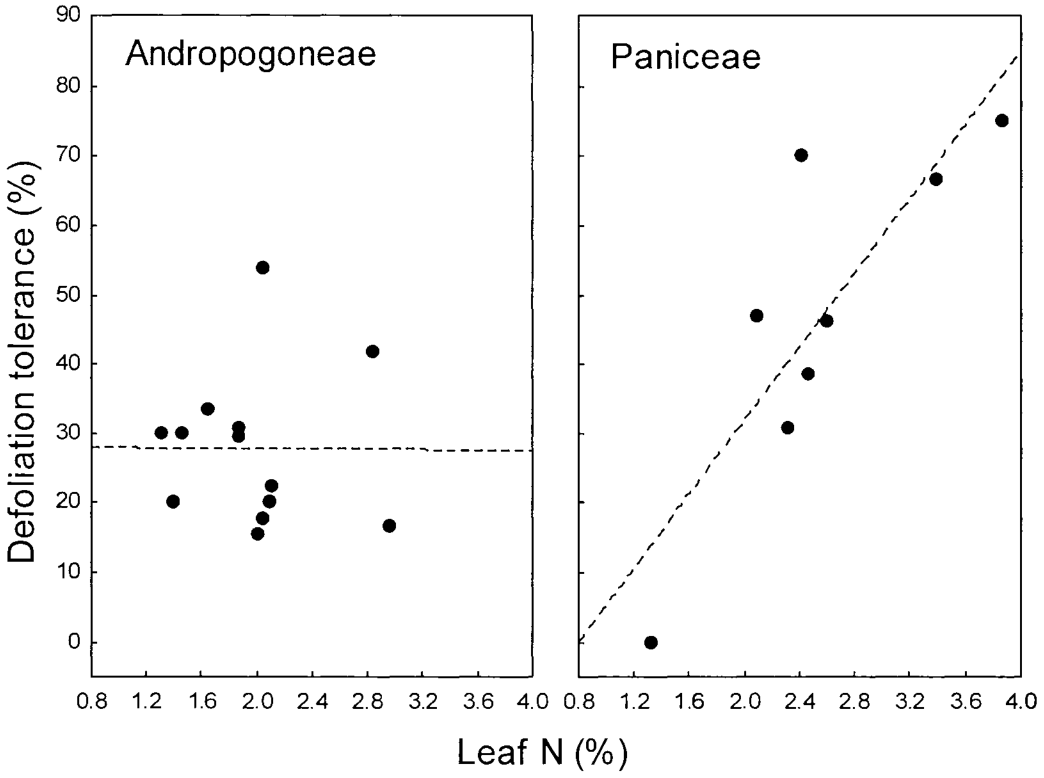

Figure 5.5. Difference in the effect of leaf nitrogen (N) on defoliation tolerance for species in the tribes Andropogoneae and Paniceae. Leaf N is for the defoliation treatment. The difference between the tribes was statistically significant according to ANCOVA (see text for details).

CHAPTER 6

Growth responses of two dominant C_4 grass species to altered water availability

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ABSTRACT

Identifying key ecophysiological traits that differ among dominant plants species and can be linked to species-specific responses to drought would improve our ability to forecast community and ecosystem responses to global climate change. The mesic grasslands of the central plains of North America are dominated by two C_4 grass species, *Andropogon gerardii* and *Sorghastrum nutans,* which purportedly differ in their tolerance of water stress. Individuals of these two species were grown in the field under rain-out shelters and subjected to wet (watered every two to three days) or dry (repeatedly subjected to wilting prior to watering) soil moisture regimes. A range of ecophysiological traits potentially important for tolerating water stress were concurrently measured. Although few traits differed between the species in the wet treatment, several traits were identified in the dry treatment that may enable *A. gerardii* to better tolerate drought. These were greater allocation to roots, reduced allocation to flowering, more rapid leaf turnover, and more rapid recovery of photosynthesis after wilting. The latter two traits may be particularly important for coping with increased variability in rainfall regimes in the future, and are consistent with recently documented responses of *A. gerardii* to experimental increases in soil moisture variability.

INTRODUCTION

The effects of global climate change on plant communities and ecosystems will be determined by responses of individual species, with the dominant species of primary importance (Smith and Knapp 2003). Similar responses among species would make changes in composition and productivity straightforward to predict, but species-specific or functional group-specific responses are more likely, rendering predictions of future productivity and diversity more complex. Identifying those ecophysiological traits responsible for species-specific responses to important environmental variables is a first step towards understanding the range of responses that can be expected for existing plant communities.

Greater variability of rainfall, leading to a greater frequency of periods of severe water stress during the growing season, is predicted for the many of the grassland regions of the world, including those of North America (Easterling et al. 2000, Houghton et al. 2001). This study investigated potential mechanisms underlying the responses of the two dominant C_4 grass species of the mesic grasslands of North America to repeated and severe reductions in soil water availability during the growing season.

Currently, the mesic grassland plant communities in the central US are dominated by a single species, *Andropogon gerardii* Vitman (big bluestem), but almost always in association with the co-dominant species *Sorghastrum nutans* (L.) Nash (Indiangrass) (Weaver & Fitzpatrick 1932, Weaver 1954). Despite the overlap in distribution, and the morphological similarity of these two species, there is evidence that *A. gerardii* is more tolerant of water stress. While the abundance of both species is positively correlated with rainfall, the above-ground productivity of *S. nutans* is greatest in the most mesic part of their shared distribution (the southern-eastern portion of the mesic grasslands), while the greatest productivity of *A. gerardii* occurs further north where mean annual rainfall is lower (Brown 1993, Epstein *et al.* 1998). Furthermore, Weaver & Fitzpatrick (1932) observed *S. nutans* only matches *A. gerardii* in abundance in the wettest parts of the prairie, as well in regularly flooded areas. Weaver (1954) reported that populations of *S. nutans* suffered greater mortality than *A. gerardii* during the severe drought of the early 1930's. Based on 15 years of data at a single site, Silletti & Knapp (2002) found that absolute cover was positively correlated with annual rainfall for *S. nutans* but not for *A. gerardii*, which was able to maintain stable cover even during dry years. Finally, a recent rainfall manipulation study by Fay *et al.* (2003) has shown that *S. nutans* was negatively impacted by increasing intervals between rainfall events (in terms of aerial cover and flowering stem density) while *A. gerardii* was again less sensitive to extended dry periods.

A wide range of traits for coping with water stress, from biochemical to morphological, have been identified in vascular plants (Chaves *et al.* 2003). Results for C_4 grasses specifically indicate as great a variety of such traits just within this functional group. In response to manipulations in water availability, co-occurring species differ in their adjustment of: the concentration of total non-structural carbohydrates (Simoes & Baruch 1991), osmotic water potentials (Knapp 1984, Barker *et al.* 1993), rates of photosynthesis and stomatal conductance (Williams & Black 1994, Baruch & Bilbao 1999, Maroco *et al.* 2000), specific leaf area (Williams & Black 1994, Kalapos *et al.* 1996, Fernandez & Reynolds 2000, Maroco *et al.* 2000), allocation to leaves and rates of leaf area expansion (Maroco *et al.* 2000, Guenni et al. 2002), and allocation to roots and root depth distributions (Simoes & Baruch 1991, Guenni *et al.* 2002). While any of these traits could be responsible for differences in water stress tolerance between *A. gerardii* and *S. nutans*, we focused on key photosynthetic parameters, leaf expansion rates and biomass allocation patterns in this study.

METHODS

Individuals of each species (referred to as *Andropogon* and *Sorghastrum* hereafter) were transplanted from local wild populations and grown in pots under rain-out shelters. These shelters form part of the Rainfall Manipulation Plot (RaMP) facility described by Fay *et al.* (2000). The pots were located in areas not affected by the RaMP sprinklers and were watered by hand. Plants were grown individually from similar-sized rhizomes harvested from the Konza Prairie Biological Station (KPBS) in the Flint Hills of northeastern Kansas, USA (39° 05' N, 96° 35' W). Pots were placed far enough apart to prevent overlap of neighbouring leaf canopies. This method was chosen to eliminate variability in plant responses caused by asymmetric below-ground competition and shading.

Plant collection

Rhizomes of *Andropogon* and *Sorghastrum* were randomly collected from two sites on KPBS. The first site consisted of both irrigated and control transects of a long-term irrigation experiment (initiated in 1991, Knapp *et al.* 2001). This site has been protected from grazing for over 20 years, but is burned annually. The second site was an upland ridge within a watershed grazed by bison (*Bos bison*) and burned only once every twenty years. At all sites, *Andropogon* is the most abundant plant species followed by *Sorghastrum*, as is typical on KPBS (Hartnett & Fay 1998). Rhizomes were collected in the second week of May 2003, after tillers with one to two immature leaves had emerged from the soil. Individual rhizomes were separated from surrounding soil, and all but the largest tiller trimmed off. They were planted individually into circular pots (60cm deep, 10.2cm inside diameter), filled with a mixture of the A and B horizon of a Irwin silty clay loam soil typical of lowland terraces and floodplains on KPBS (Ransom *et al.* 1998). These are fertile soils, with mineralization rates in the range of 1 to 4 g N m $^{-2}$ per growing season (Blair *et al.* 1998), although mineralization rates were probably higher in this study due to soil disturbance. The soil was initially sieved through 1 cm^2 mesh to remove stones and large pieces of litter, and compacted to have a uniform bulk density of approximately 0.98 g cm^{-3}. Fiberglass mesh was used as a base for the pots. The transplants were grown in full sunlight and watered at least every two days for approximately 5 weeks before experimental treatments were imposed.

Our original goals of the experiment included an assessment of differences between potential ecotypes of *Andropogon* collected from different habitats (the irrigated, control and grazed sites mentioned above). The original ecotypic aspect of the study is responsible for the unbalanced sampling design with regard to far greater numbers of *Andropogon* than *Sorghastrum* collected. Since no significant differences were found between the putative *Andropogon* ecotypes, only differences between *Andropogon* and *Sorghastrum* are reported here.

Initial growth

To estimate initial rates of growth, the tiller from each transplanted rhizome was clipped to a height of 3cm approximately four weeks after transplanting. Any additional tillers that emerged subsequent to transplanting were included in this harvest. Harvested biomass was oven-dried to a constant mass and weighed. Based on the leaf area of regrowth following this initial harvest, subsets of individuals of similar size were selected for the watering experiment (138 for *Andropogon*, 20 for *Sorghastrum*).

Watering treatments

The pots were moved under three permanent rain-out shelters in early June 2003. Individuals were randomly assigned to one of two watering treatments: watered to field capacity every two to three days (wet treatment) or only watered after plants had been completely wilted (i.e. all leaves folded or rolled) for 3 to 4 days (dry treatment). Based on the initial leaf area measurements (see below), individuals of each species were assigned to one of three initial groups. The first group contained individuals with the largest leaf area (n = 17), the second intermediate (n = 79), and the third group with the smallest leaf area produced (n = 62). Watering treatments were initiated on the 18th, 20th and 24th June 2003 for the first, second and third initial groups, respectively. The purpose of delaying the initiation of the treatments by group was to minimize differences in size (within each species) at the time that the watering treatments were imposed.

Gas exchange

Measurements of leaf-level net CO_2 assimilation (A), stomatal conductance (g_s), transpiration (E), leaf vapor pressure deficit (VPD), and the ratio of intercellular to ambient CO_2 concentration (c_i/c_a) were made on a subset of individuals (7 replicates per treatment for *Andropogon*, and 2 (wet) and 5 (dry) replicates for *Sorghastrum*)). These individuals were measured at approximately weekly intervals over the course of the experiment. Measurements were taken on the youngest fully expanded leaf (one leaf per individual) using a LICOR 6400 infrared gas analyzer (LI-COR, Lincoln, Nebraska) with a 6400-02B LED light source cuvette. Measurements were restricted to warm days with little or no cloud cover, and were taken between 12h00 and 16h00. Light levels were set to 1500 μmol m^{-2} s^{-1} PAR. Temperature was set to 35°C but as the cuvette could not regulate temperature completely on particularly cool or warm days, leaf temperature at measurement varied from 31 to 38°C. Relative humidity (RH) was maintained within 5% of ambient (measured at the start of each measurement period).

Leaf area and wilting

Canopy leaf area was determined by measuring the length and maximum width of all leaf blades on an individual. This was done for all individuals at the start of the watering treatment. Thereafter, the same subset of individuals used for the gas exchange measurements were measured at approximately weekly intervals and at harvest. Leaves were grouped into four categories to improve the accuracy of this method: fully expanded mature leaves with ligule development, mature leaves which had previously been clipped, immature leaves with at least half the length of the leave blade expanded, and emerging leaves with more than half the blade still rolled. To estimate leaf area from these dimensions, direct measurements were also taken on a subsample of blades from a few additional individuals harvested at the start of the experiment, and at the final harvest. Leaf area for the subsampled blades was measured with a leaf area meter (LI-3100, LiCor, Lincoln, Nebraska). As the projected area of the emerging leaves was approximately rectangular in shape, area was calculated simply as length x width. For the other three categories, area was regressed against blade length and blade width, using a separate multiple regression for each category (13 to 96 leaves per regression). All variables were ln transformed to account for increasing variance in area with increasing length and width, and linearity was confirmed from plots of residuals. All regressions were significant at $p < 0.001$, and r^2 values ranged from 0.88 to 0.99.

For the dry treatment, dates were recorded when the leaves of an individual first began to fold or roll, and when the individual was completely wilted (all leaves folded or rolled). The number of days to wilting was then calculated as the number of days from the last watering until complete wilting, for each wilting event experienced by each individual.

Biomass

Plants were harvested after 42 to 43 days of growth in the wet treatment, and 63 to 72 days in the dry treatment. Harvest dates differed to allow individuals to reach a similar size (for the correct interpretation of biomass allocation patterns, individuals of similar size are required, in order to it separate treatment effects from ontogenetic drift - Coleman *et al.* 1994). Harvested plants were separated into senesced material, roots (washed with water and sodium hexametaphosphate), rhizomes, sheaths and culms, and leaf blades. Allocation to each component, with the exception of "senesced", was calculated as a mass fraction, using total live mass (e.g. blade mass fraction = blade/total live). The contribution of senesced material was calculated as senesced/(total live + senesced). Specific leaf area (SLA) was calculated as canopy green leaf area/leaf blade mass at harvest, and leaf area ratio (LAR) as canopy green leaf area/total live mass.

Statistical analysis

Gas exchange data were analyzed separately for the wet and dry treatment. For the wet treatment A, g_s, and VPD were analyzed with a repeated measures ANOVA for each variable (with week of measurement as the repeated measure and species as a main effect). Models with various covariance structures were fitted using SAS v8.01 (SAS Institute Inc, Cary), and the best fit selected on the basis of the corrected Akaike's Information Criteron (AIC_c). For the dry treatment, data were calculated as a percentage of the wet treatment mean. This analysis was limited to measurements taken before and shortly after the first period of wilting, as differences in the time to wilting between species resulted in watering schedules becoming progressively offset thereafter. The number of days prior to and subsequent to the first wilt was calculated for each individual measured. Data were then grouped into 2 to 4 day periods before and after wilting, as well as for the period when all individuals were completely wilted when measured. As measurements for an individual did not occur in each time period, data for each period were treated as a random sample of the subset of individuals measured and analyzed with a factorial ANOVA (with species and time period as main effects). Differences between species for particular time periods were subjected to planned comparison tests using Statistica v6.1 (Statsoft Inc., Tulsa). Differences between species for the slope of A versus g_s, and g_s versus VPD were tested using ANCOVA, separately for each treatment. For these analyses all data collected during the experiment were used, and data collected during the first three days of the water stress treatment were used in the wet treatment analysis as plants were not stressed at this time. For A versus g_s a polynomial term (g_s^2) was included with the covariate to account for non-linearity.

Biomass allocation variables were analyzed separately. As samples sizes were much greater for *Andropogon* than *Sorghastrum*, variances were generally larger for the latter. For some variables (rhizome mass fraction, senesced mass fraction, SLA and LAR), ln or square-root transformation were sufficient to homogenize variance and created a normal distribution of residuals. In these cases, data from both watering treatments were analyzed together, using a factorial ANOVA (with species and watering treatment as main effects). For the remaining variables, the effect of the watering treatment was tested separately for each species using Student's t-tests.

RESULTS

Initial growth

In the initial four weeks following transplanting, *Sorghastrum* grew faster than *Andropogon* (mean ± SE dry mass of harvested biomass: *Andropogon* = 0.046 ± 0.002g, *Sorghastrum* = 0.058 ± 0.007g , $t = -2.27$, $p = 0.03$). Leaf area production in the week following the initial harvest exhibited the opposite pattern, as *Andropogon* canopy leaf area was significantly greater for the initial leaf area measurements (mean ± SE: *Andropogon* = 9.6 ± 0.30cm^2, *Sorghastrum* = 7.4 ± 1.61cm^2, $t = 4.74$, $p < 0.01$).

Gas exchange

In the wet treatment, A was significantly higher for *Sorghastrum* ($F_{1,11.6} = 4.99$, $p = 0.046$; Fig 6.1a) as was g_s ($F_{1,10.6} = 11.48$, $p < 0.01$; Fig 6.1c). There was a slight, non-significant decline in both parameters over the first 5 weeks of growth for both species, with no interaction of species and time. In the dry treatment, A declined slowly for both species as the first wilting point approached, with mean rates of about 80% of the wet treatment mean 4 to 6 days prior to complete wilting (Fig 6.1b). Planned comparisons indicated that reductions in A were significantly greater for *Andropogon* 1 to 3 days prior to wilting. However, A recovered more rapidly for *Andropogon* following rewatering, with rates of about 100% of the wet treatment mean 2 to 3 days after watering. A similar pattern was observed for g_s, with *Andropogon* recovering to the wet treatment mean within 3 days of rewatering, and *Sorghastrum* only recovering to about 70% (Fig 6.1d.).

VPD did not vary significantly across these periods, nor between species for any one period (data not shown).

The similar responses in A and g_s to declining soil water availability were reflected by the tight coupling between A and g_s for data from both treatments and both species (Fig 6.2). ANCOVA revealed no differences between species in the slope of A versus g_s for either treatment, indicating no differences in intrinsic water use efficiency (wet treatment: $F_{1,65} = 0.20$, $p = 0.65$; water stress treatment: $F_{1,116} = 0.061$, $p = 0.81$). For the dry treatment data, c_i/c_a did not show any increase as plants approached wilting point (Fig 6.3). For open leaves there was actually a significant negative correlation of c_i/c_a with days to wilting for both species (*Andropogon*: $r = -0.31$, $p < 0.01$; *Sorghastrum*: $r = -0.52$, $p < 0.01$), revealing a draw-down of intercellular CO_2 created by reduced stomatal conductance. This trend was reversed once leaves began to roll (for *Sorghastrum*) or once fully folded (for *Andropogon*), indicating a reduction in demand for CO_2 and therefore reduced photosynthetic capacity. For fully folded/rolled leaves, c_i/c_a rose to greater values in *Andropogon*, indicating a greater reduction in photosynthetic capacity.

In the wet treatment, the higher g_s for *Sorghastrum* occurred over the full range of VPD encountered during the study ($F_{1,68} = 17.7$, $p < 0.01$; Fig 6.4a) and both species showed a similar, yet slight decrease in g_s with increasing VPD ($F_{1,68} = 7.73$, $p < 0.01$). In contrast, in the dry treatment g_s was not greater, on average, for *Sorghastrum* (Fig 6.4b). The slope of g_s against VPD was again similar for both species ($F_{1,119} = 0.386$, $p < 0.01$) but steeper than in the wet treatment.

Leaf area and wilting

Leaf canopy area increased exponentially over the first 28 to 42 days in the wet treatment. During this period, rates of increase (approximately 9% per day) did not differ significantly between species. In the dry treatment, leaf canopy expansion prior to the first leaves folding/rolling was not significantly lower than in the wet treatment, and again did not differ between species.

Leaves senesced at a similar rate for both species as leaf number increased in the wet treatment, and by the end of the experimental period (42 to 43 days of growth) the proportion of all leaves produced that had senesced was similar (*Andropogon* = 0.141, *Sorghastrum* = 0.143). In the dry treatment, *Sorghastrum* individuals wilted sooner than *Andropogon*, over the first three wilting events ($F_{1,49.4} = 165$, $p < 0.01$; Fig 6.5). The number of days from watering until wilting declined by almost half for both species for the second wilting period, and then only slightly more for the third wilting period. The overall decline was significant for both species combined ($F_{2,48.4} = 284$, $p < 0.01$). As a result of wilting more rapidly, *Sorghastrum* individuals had to be watered more frequently than *Andropogon* over the course of the experiment. Mean number of waterings was 2.5 for *Andropogon*, and 3.1 for *Sorghastrum*. The first wilting period caused a significant increase in the proportion of leaves senesced ($F_{1,49} = 9.24$, $p < 0.01$), with a similar increase for both species (Fig 6.6a). This proportion then remained fairly constant prior to, and after, the second period of wilting. Across all four measurement periods, the proportion of leaves senesced was significantly higher for *Andropogon* ($F_{1,49}$

= 8.29, $p < 0.01$). *Andropogon* also had a higher proportion of live leaves in the emerging category (Fig 6.6b), indicating a greater turnover of leaves. The proportion of leaves emerging did not decline across the first two wilting periods for *Andropogon*, but did for *Sorghastrum*, and was significantly greater for *Andropogon* across all periods ($F_{1,48}$ = 12.5, $p < 0.01$).

At the time of harvest, SLA was greater for *Andropogon*, while LAR did not differ between the species in the wet treatment (Table 1). In the dry treatment, SLA was significantly lower for *Andropogon* but not for *Sorghastrum*, while both species showed greater LAR compared to the wet treatment (Table 1).

Biomass allocation

Final biomass was similar for *Andropogon* and *Sorghastrum* in the wet treatment (mean dry mass for *Andropogon* = 19.4 ± 0.67g, *Sorghastrum* = 18.6 ± 1.3g). Allocation of live biomass to roots was also similar, while allocation to sheaths and culms was greater for *Andropogon*, and allocation to blades and to rhizomes greater for *Sorghastrum* (Table 1). The proportion of total biomass produced consisting of senesced material was significantly greater for *Sorghastrum.* In the dry treatment, allocation to leaf blades was higher than in the wet treatment for both species, with *Sorghastrum* again allocating more than *Andropogon*. Allocation to rhizomes was significantly greater than in the wet treatment for *Andropogon*, but not for *Sorghastrum. Andropogon* showed similar allocation to roots, while *Sorghastrum* showed significantly lower allocation. The proportion of total biomass consisting of senesced material was significantly greater for *Andropogon* than in the wet treatment, but not statistically different for *Sorghastrum*.

In the wet treatment 23% of *Andropogon* individuals had initiated inflorescences by harvest. The *Andropogon* individuals that flowered had lower allocation to rhizomes than those that had not yet flowered (0.027 versus 0.045; $t_{7,29}$=-3.89, $p < 0.01$) and there was a negative correlation between allocation to rhizomes and allocation to culms and sheaths ($r = -0.38$, $p < 0.01$). In the dry treatment, only 3 *Andropogon* individuals (5%) had initiated inflorescences by harvest, and biomass allocation to sheaths and culms was significantly lower than in the wet treatment (Table 1). While many *Sorghastrum* individuals began producing culms, no inflorescences were recorded in either treatment, and allocation to sheaths and culms was similar.

DISCUSSION

The similar growth of *Andropogon* and *Sorghastrum* in the wet treatment did not reveal any traits that could explain the greater dominance of the former in mesic grasslands. Both species expanded their canopy leaf area at a similar rate and produced a similar amount of biomass. Slightly higher SLA of *Andropogon* was balanced by slightly higher allocation to leaf blades by *Sorghastrum*. *Sorghastrum* actually exhibited two traits which could allow greater productivity in the absence of water stress: higher A per unit leaf area and greater allocation to rhizomes. Higher A for *Sorghastrum* was also found by Silletti & Knapp (2001) in irrigated transects on KPBS. The higher g_s shown by *Sorghastrum* can explain, at least partly, the greater *A*. Additional photosynthate gained by higher *A* did not result in faster canopy expansion for *Sorghastrum* in this experiment, but may have been converted to storage compounds and thus greater allocation to rhizomes. Greater allocation to storage could translate into faster initial growth at the start of the growing season, when stored photosynthates are the sole source of energy for spring leaves. The faster initial growth indicated by the initial harvest supports this idea, as do the results of Cuomo *et al.* (1998) who found that etiolotated growth by *Sorghastrum* was less affected by regular clipping than for *Andropogon*. Faster initial growth at the start of the growing season, in the absence of light competition, could lead to greater shading of neighbours and eventually greater aerial cover. Greater allocation to rhizomes may also lead to greater vegetative reproduction and thus more rapid population expansion. This is consistent with observations of *Sorghastrum* producing new tillers throughout the growing season, with *Andropogon* tillers only emerging in the spring (McKendrick *et al.* 1975). Both greater allocation to rhizomes and higher *A* could explain *Sorghastrum's* increased absolute cover under conditions of annual spring burning on KPBS (Gibson & Hulbert 1987, Hartnett *et al.* 1996, Silletti & Knapp 2002) in contrast to a lack of response by *Andropogon*. Similarly, these traits likely contribute to the greater above-ground productivity of *Sorghastrum* monocultures, relative to *Andropogon*, observed in wet sites or in wet years (Hadley & Kieckhefer 1963, Cuomo *et al.* 1996).

The far greater abundance of *Andropogon* in the majority of mesic grassland communities suggests that significant water stress occurs frequently enough to nullify advantages *Sorghastrum* may have during periods of non-limiting soil moisture.

However, on KPBS a long-term irrigation experiment designed to eliminate all water stress (Knapp *et al.* 2001) had not yet resulted in an increase in the density of tillers of *Sorghastrum* after 13 years (Swemmer, unpub. data). Other factors may negate the ability of *Sorghastrum* to grow as rapidly as *Andropogon* in the absence of water stress. Gibson & Skeel (1996) found that both *A* and total biomass were reduced for *Sorghastrum* when grown in competition with other prairie species. Responses of the two species to nitrogen fertilization on KPBS suggest that *Andropogon* may have higher nitrogen use efficiency (Silletti & Knapp 2001). Finally, differences in recruitment may be important - Mueller & Weaver (1942) found lower rates of seed germination for *Sorghastrum* compared to *Andropogon*, and Weaver & Mueller (1942) found many seedlings of *Andropogon,* but few of *Sorghastrum*, following the severe drought of the 1930's.

In the dry treatment, *Sorghastrum* pots received more water over the course of the experiment due to more frequent wilting. Therefore, measures of growth rates are not comparable between the species. However, the differences in allocation and leaf-level traits point to key differences in their ability to adjust physiologically and morphologically to repeated bouts of water stress (i.e. wilting events of 3 to 4 days duration). Net CO_2 assimilation (*A*) declined at a similar rate in both species as water stress developed. Initial reductions appear to have been from stomatal limitations only, as indicated by no increase in c_i/c_a values until leaves began folding/rolling. The sensitivity of stomatal conductance to evaporative demand was also similar for both species during this period. The pattern of similar photosynthetic responses prior to severe stress are supported by the results of Polley *et al.* (1992) who found no differences in photosynthetic parameters between these two species under moderate water stress in the field. However, this does not appear to be the case for all co-occurring C_4 species. With increasing water stress, Guenni *et al.* (2004) found differences in reductions in A, g_s and A versus g_s amongst five C_4 species of *Brachiaria*, while Williams & Black (1994) found similar differences between *Heteropogon contortus* and *Pennisetum setaceum*. With more severe water stress some differences in photosynthetic responses were exposed, in this study, with reductions in photosynthetic capacity apparently greater for *Andropogon*.

The similar patterns of reductions in photosynthesis did not persist following wilting, as A and g_s recovered to maximum levels more rapidly for *Andropogon*. More rapid recovery of g_s for *Andropogon* was also shown by Martin *et al.* (1991), who found that g_s was nearly doubled immediately following a large rainfall event which ended a severe drought for this species, while it did not increase significantly for *Sorghastrum*. The recovery of A does not appear to have resulted from greater tolerance of *Andropogon* leaves to water stress. Instead, the higher proportion of leaves senescing with wilting, and the greater number of leaves emerging following rewatering resulted in a canopy of younger leaves in *Andropogon*. This resulted in the gas exchange measurements following rewatering being taken mainly on new leaves for *Andropogon,* but on older, recovering leaves for *Sorghastrum*. The post-wilting emergence of *Andropogon* leaves was rapid enough to produce a canopy leaf area equivalent to *Sorghastrum* by the end of the experiment (indicated by similar final biomass and LAR). Greater leaf turnover thus enabled *Andropogon* to effectively avoid the effects of severe water stress on its leaves. Furthermore, it enabled an overall reduction in SLA, not observed in *Sorghastrum*, with new cohorts of leaves potentially better acclimated to tolerate future water stress. Reduced SLA with water stress is a common response in herbaceous plants, which may result from greater allocation to structural tissues and therefore less sensitivity to low leaf water potentials (Chaves *et al.* 2003).

The benefits of higher leaf turnover following water stress would be greater if accompanied by the retranslocation of nutrients out of the leaves before senescence (Chaves *et al.* 2003). Retranslocation of leaf nitrogen, leading to substantial increases in carbon gain following alleviation of drought stress, has been shown for *Andropogon* (Heckathorn *et al.* 1997). In this experiment, larger reductions in A in *Andropogon* 1 to 3 days prior to wilting, as well as the apparently greater reduction in photosynthetic capacity of folded leaves, may have resulted from the onset of nutrient retranslocation. While *Sorghastrum* undoubtedly also retranslocates nitrogen, it was found to have a higher proportion on nitrogen in shoots (relative to *Andropogon*) during a year of below-average precipitation (Adams & Wallace 1985).

Rapid senescence of leaves at the onset of water stress is a trait of many drought tolerant species (Chaves *et al.* 2003), but its potential ecological significance has not been shown for other C_4 grass species. While differences in the recovery of A between co-occurring C_4 species were also reported by Guenni *et al.* (2004), it was not determined whether this resulted from leaf turnover, or differences in the tolerance of water stress of surviving leaves.

Greater leaf longevity in *Sorghastrum* during periods of water stress may be linked to reductions in photosynthetic capacity in rolled leaves and this reduction persisted for a number of days following rewatering. When water stress is not severe, these reductions may not be of much significance, and could even be less than those resulting from leaf aging in well-watered plants, as found for another C_4 grass (*Panicum maximum*) by Ludlow & Ng (1974). However, the maintenance of more live leaves during a period of water shortage also incurs the cost of greater whole-plant transpiration, increasing the risk of severe water stress.

In addition to greater leaf turnover following water stress, *Andropogon* displayed two other morphological traits that may explain its greater abundance in areas with more frequent water stress. Firstly, *Andropogon* allocated more to roots than *Sorghastrum* in the dry treatment. Greater allocation to roots may allow *Andropogon* to delay or avoid periods of mild water stress resulting from the drying of upper soil layers. This is consistent with the results of root excavations reported by Weaver (1954), which revealed greater rooting depths for *Andropogon*. It is surprising that this difference resulted from decreased allocation to roots by *Sorghastrum*, rather than increased allocation by *Andropogon*, as the latter response has been found for a wide range of drought tolerant plants (Fernandez & Reynolds 2000, Poorter & Nagel 2000). Decreased allocation to roots with water stress was also observed for other C_4 grass species from mesic environments (Simoes & Baruch 1991, Guenni *et al.* 2002).

The third potentially important trait shown by *Andropogon* was reduced allocation to flowering. This pattern has also been observed in the field, with *Andropogon* populations producing no inflorescences at all during drought years on KPBS (Knapp 1984; Swemmer, pers. obs.). While *Sorghastrum* did not flower successfully on either treatment, flowering culms were initiated in both treatments, resulting in similar allocation to sheaths and culms at harvest. This indicates that individuals of *Sorghastrum* were attempting to flower in the dry treatment (and may have if allowed to grow for a longer period). The reduction in allocation to sheaths and culms shown by *Andropogon* was balanced by increased allocation to rhizomes. Resources therefore appear to have been diverted away from flowering, and into greater storage.

Conclusion

When grown without water stress, no traits that could explain the greater abundance of *Andropogon* than *Sorghastrum* in mesic environments were identified. In contrast, repeated bouts of water stress revealed a number of traits relating to water use that may explain the greater abundance of *Andropogon* in the drier regions of mesic grasslands, as well as its superior ability to cope with experimentally increased variability in soil moisture. These were a greater turnover of leaves during wet-dry cycles leading to more rapid recovery of photosynthesis following wilting, greater allocation of biomass to roots, and a reduction in allocation to flowering (relative to unstressed controls). While *Sorghastrum* was able to maintain more live leaves during the periods of water stress in this experiment, the traits exhibited by *Andropogon* are likely to give this species an advantage when stress is prolonged or more frequent. These results provide an indication of which traits may be important in predicting the responses of other grass species to future changes in rainfall, and indicate that the more extreme drought periods forecast for the mesic grasslands of the central US will likely increase the dominance of *Andropogon*.

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TABLES AND FIGURES

Table 6.1. Leaf area and biomass allocation of *Andropogon* and *Sorghastrum* for the wet and dry treatments at the final harvest. Allocation variables are expressed as fractions of total live biomass, with the exception of "senesced" which is the fraction of senesced material divided by total biomass produced. Values are least square means with SE in parentheses. Significant differences ($p < 0.05$) for each variable are indicated by superscripts a and b for variables where both the wet and dry treatment data were analyzed together in a factorial ANOVA. Where watering treatment comparisons were conducted separately for each species (leaf blades, sheaths and culms, and roots), significant differences are indicated with superscripts a and b (wet treatment) or x and y (dry treatment).

		Andropogon		Sorghastrum	
		Wet	Dry	Wet	Dry
Leaf area	**SLA (m^2 kg^{-1})**	12.3 (0.43)^{a}	10.4 (0.50)^{b}	11.01 (0.47)^{b}	10.1 (0.74)^{b}
	LAR (cm^2 g^{-1})	22.6 (1.7)^{a}	30.1 (1.8)^{b}	23.9 (1.7)^{a}	27.6 (2.9)^{b}
Biomass allocation	**Leaf blades**	0.18 (0.009)^{a}	0.27 (0.010)^{b}	0.22 (0.009)^{x}	0.30 (0.011)^{y}
	Sheaths and culms	0.33 (0.025)^{a}	0.22 (0.018)^{b}	0.25 (0.024)^{x}	0.23 (0.015)^{x}
	Rhizomes	0.044 (0.003)^{a}	0.074 (0.004)^{b}	0.094 (0.013)^{b}	0.088 (0.014)^{b}
	Roots	0.45 (0.020)^{a}	0.44 (0.015)^{a}	0.44 (0.016)^{x}	0.38 (0.024)^{y}
	Senesced	0.040 (0.004)^{a}	0.059 (0.006)^{b}	0.060 (0.014)^{b}	0.045 (0.012)^{ab}

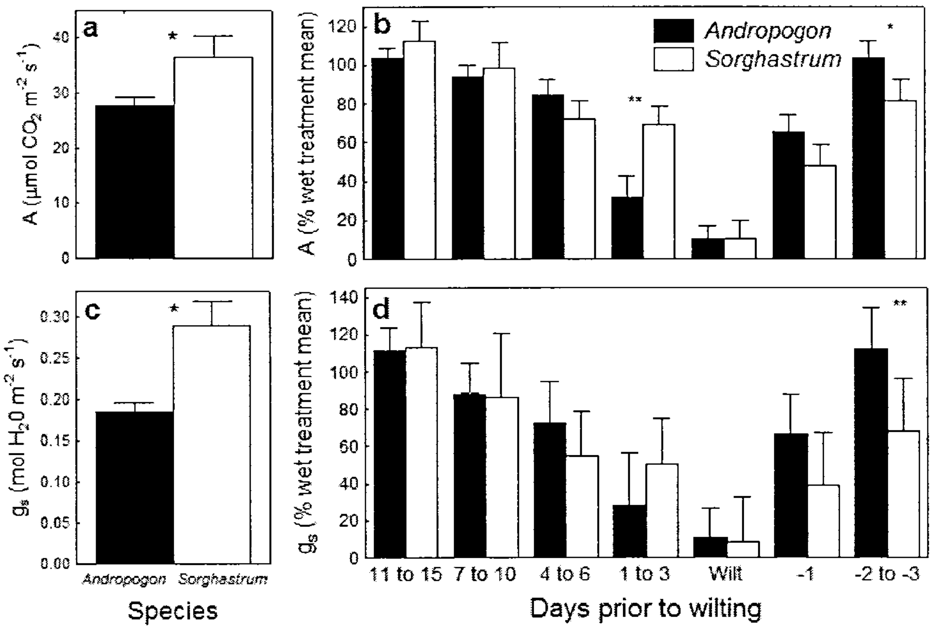

Figure 6.1. a) Net CO_2 assimilation (*A*) and c) stomatal conductance (g_s) for the wet treatment, averaged over 5 weeks of measurement. b) A and d) g_s as percentage of wet treatment means before and after the first wilt for the dry treatment. Negative values on the x axis indicate days following rewatering after the first wilt. Bars are least square means (+1 SE). Asterisks indicate significant differences between species, based on planned comparison tests: ** $p < 0.05$, * $p < 0.10$.

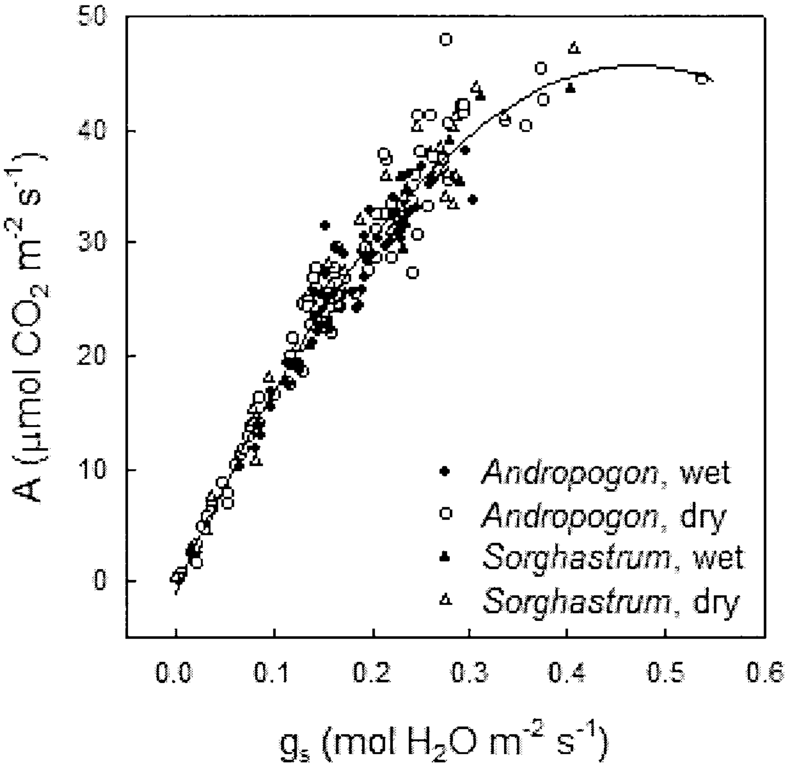

Figure 6.2. Net CO_2 assimilation (A) versus stomatal conductance (g_s) for *Andropogon* and *Sorghastrum* for both the wet and dry treatments. A polynomial regression provided a significant fit to all data combined for both species: $A = -1.02 + 198(g_s) - 210(g_s)^2$; $r^2 = 0.90$, $p < 0.01$.

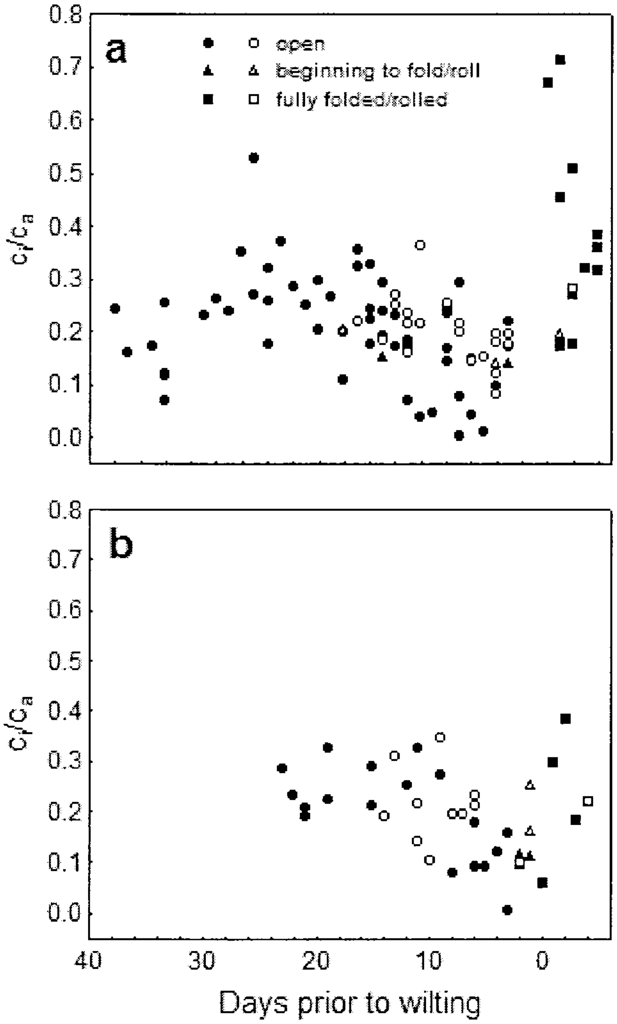

Figure 6.3. The ratio of intercellular to ambient CO_2 concentration (c_i/c_a) versus the number of days until wilting for a) *Andropogon* and b) *Sorghastrum*, for the dry treatment. Data are for measurements taken prior to the first wilt (solid symbols), and subsequent wilts (open symbols) for leaves either fully open, beginning to fold or roll and completely folded or rolled.

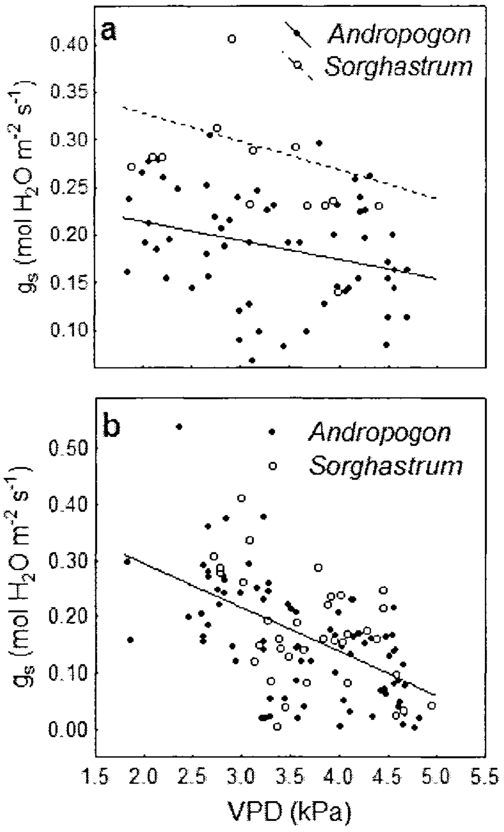

Figure 6.4. Stomatal conductance (g_s) versus leaf vapor pressure deficit (VPD) for *Andropogon* and *Sorghastrum* for the wet treatment (a) and dry treatment (b). For the wet treatment, g_s was significantly higher for *Sorghastrum*, averaged across VPD. The linear effect of VPD on g_s did not differ between species for either treatment (therefore the slopes of the fitted lines in a) are not significantly different). Trend lines are derived from ANCOVA analyses (see text for details): a) *Andropogon*: $g_s = 0.245 - 0.020(\text{VPD})$, *Sorghastum*: $g_s = 0.388 - 0.040(\text{VPD})$, b) both species combined: $g_s = 0.45 - 0.078(\text{VPD})$.

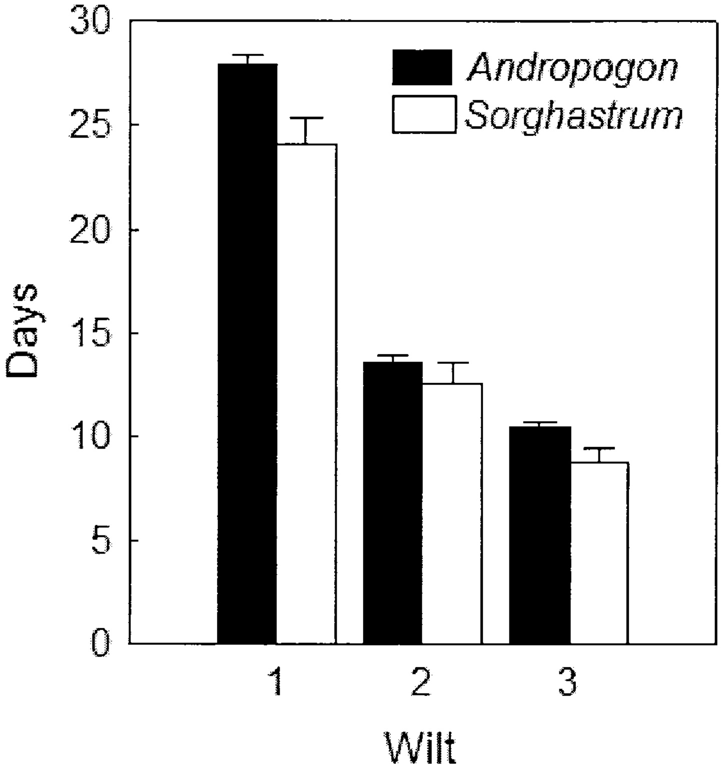

Figure 6.5. The mean number of days to wilting for the first three wilting events for *Andropogon* and *Sorghastrum* in the dry treatment. Days to wilting declined significantly for each event and was significantly greater for *Andropogon* over all three events. Error bars show 1 SE.

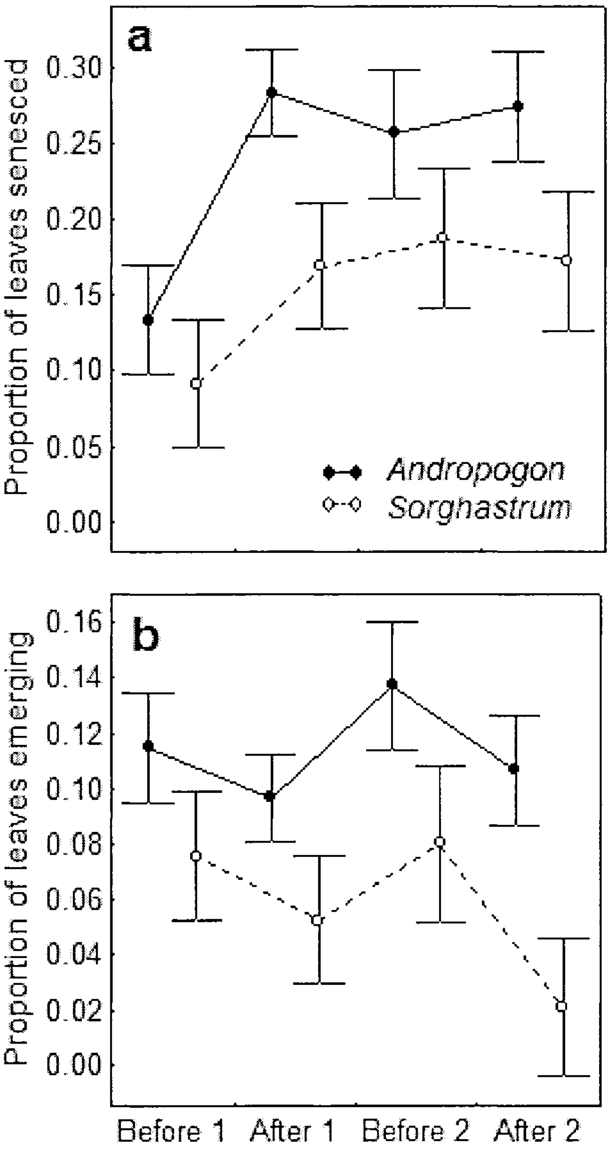

Figure 6.6. The proportion of total leaves senesced (a) and the proportion of live leaves emerging (b), before and after the first two wilting events for individuals subjected to the dry treatment. Points are least square means and error bars show 1 SE.

CHAPTER 7

Summary

The overall objectives of this dissertation, to determine the effects of intra-seasonal variation in precipitation and grass community composition on the above-ground net primary productivity (ANPP) of grasslands, were addressed using existing long-term data, a multiple-site, multiple-year field experiment and a short-term pot experiment. The research focused on the short-term, above-ground growth of perennial C_4 grasses. Analyses were conducted using data from ten grassland and savanna sites, on two continents. The aim of working at multiple sites was not to compare any of the sites (or the two continents). Rather, the range of sites provides a certain degree of confidence in extrapolating the results to other C_4 grass communities. While the effect of grazing was not of primary interest at the outset, the effect of defoliation was incorporated in the field experiment, and was found to have an important interaction with the effect of community composition.

The role of intra-seasonal variation in precipitation

Access to long-term data for three southern temperate grasslands provided an opportunity test whether the distribution of precipitation within a growing season can affect ANPP independently of total precipitation. Calculation of simple metrics to quantify intra-seasonal variation in precipitation revealed this can have large effects on ANPP but that these effects vary between sites (**chapter 2**). At two sites, intra-seasonal variation in precipitation clearly affected ANPP more than seasonal precipitation totals. The size and number of precipitation events in a growing season predicted ANPP better than the amount of precipitation at the driest of the sites, while the average interval between events was the best predictor at the wettest site. The site with intermediate rainfall was not strongly affected by intra-seasonal variation in precipitation, and productivity responded the most to total precipitation.

While it was clear that differences in species composition between sites were not responsible for the observed differences in ANPP-precipitation relationships, a greater range of sites would be required to determine exactly which site-specific factors were important. It was speculated that differences in soil texture and depth are important, as these determine how much precipitation is stored between precipitation events, and how much is lost to run-off and evapotranspiration. Deep soils with high rates of infiltration are likely to provide a buffer against intra-seasonal variation in precipitation (in the form of storage of soil water). Grasslands on these soils were therefore predicted to be least affected by a changes in precipitation patterns forecast by global climate change models (namely larger but less frequent precipitation events). In contrast, shallow soils are more likely to dry up right down the profile between precipitation events, increasing the frequency of water stress, and resulting in a relatively strong effect of the length of intervals between precipitation events.

The role of species composition

The results of the field experiment presented in **chapters 3** and **4** indicated that many co-occurring species respond differently to intra-seasonal variation in precipitation. It was clear that co-occurring species did not have similar intra-seasonal patterns of above-ground growth, but also that, for the majority of species, short-term growth was not determined by recent rainfall (**chapter 3**). Rather growth rates appeared to be a more a function of soil water availability at various depths, as well as unidentified seasonal growth constraints that restrained growth independent of water availability (**chapter 4**). Furthermore, co-occurring species appeared to differ in terms of the depth of that water was primarily utilizated and the effect of seasonal growth constraints, indicating that C_4 grasses should not be considered as a single functional group in terms of water relations.

It was argued that the utilization of deep soil water enables more efficient use of precipitation in wet years, but brings the risk of more severe water stress in dry years, or years when the distribution of precipitation events favors run-off and evaporation over infiltration (at least in ecosystems where winter precipitation does not make a significant contribution to soil water content at the start of the growing season). Shallow-rooted species would not be affected by a lack of deep infiltration but must contend with the more variable supply of water in the upper soil, as revealed by soil water measurements. These species were therefore predicted to be less affected by drought, as there will always be some wetting of the upper soil. In addition, many of these species only required a short duration of soil water availability to grow to peak biomass and flower.

A potential implication of the results of the field experiment is that the response of ANPP to changes in precipitation patterns will both cause changes in community composition, and be altered by changes in community composition. The relationship between ANPP and precipitation therefore appears more complex than most long-term studies suggest, and accurate prediction of ANPP may require knowledge of species-specific responses to both soil water availability and various (unidentified) seasonal growth constraints.

However, the effects of changes in species composition were not directly measured in the field experiment, and these results should be treated with caution. It is possible that species-specific patterns of water-utilization change in years with precipitation patterns not encountered during the 3 years of the field experiment. Furthermore, species-specific responses could be modified by changes in community composition. For example, the removal of a highly-competitive, dominant species may allow other species to produce more in response to rainfall events, or to utilize soil water at a deeper depth. The relevance of such caveats is revealed by comparing the results of the long-term data analysis (**chapter 2**) and the field experiment (**chapters 3** and **4**): the results of the latter results contradict one of the results of the former, namely that changes in species composition within a site do not alter the response of ANPP to intra-seasonal variation in precipitation. This paradox indicates that while co-occurring species may well differ in their short-term growth responses to intra-seasonal variation in water availability, such differences may not necessarily be large enough, or consistent enough, to result in meaningful differences in ANPP-precipitation relationships in the long-term.

The role of grazing

While grazing is known to affect species composition in the long term, the results of the field experiment (**chapters 3** and **4**) indicated an important short-term effect. Regular defoliation greatly reduced the variation in intra-seasonal growth rates between species, and reduced differences in soil water utilization. At least some of the seasonal growth constraints were eliminated by defoliation, while changes in allocation below-ground are the most likely cause of the observed change to more similar, shallow depths of soil water use amongst species. Frequent, non-selective grazing was therefore predicted to constrain productivity responses to precipitation at the ecosystem level, with more consistent growth through the growing season, and a weaker linear effect of annual precipitation on ANPP. Furthermore, changes in species composition were predicted to be of less significance in heavily-grazed systems.

Functional groups and predictive traits

The final two objectives of the dissertation were to determine whether grass species can be grouped according to growth responses to precipitation and defoliation, and what traits define such groups. A large variety of functional groups were evident from the results of the field experiment (**chapter 5**). However, no meaningful association between functional responses was found, and species could not be grouped into broader functional groups that would simplify predictions relating to community composition. This indicates large functional diversity within the C_4 grasses, and may partly explain the large diversity of these species in some systems. Three functional categories, relating to the depth of soil water use, vulnerability to seasonal growth constraints and changes in soil water utilization with defoliation were defined, and suites of easily-measured traits were identified that could assign species to these groups with reasonable accuracy. A fourth functional response, defoliation tolerance, was not adequately predicted by any set of traits. Two traits generally associated with faster-growing species (leaf nitrogen concentration and specific leaf area) provided the best indication of defoliation tolerance.

While various traits identified were only considered as predictive and not functional, they do provide a set of traits worth investigating for functionality, i.e. some of the traits identified may actually be responsible for functional differences between species, and may underpin eco-physiological mechanisms responsible for these differences. The pot experiment attempted to identify such mechanisms, by measuring relevant traits on two species considered to differ in their tolerance of water stress (**chapter 6**). While there was no overlap between the traits identified in this experiment and the predictive traits identified in the field experiment, it was found that greater tolerance of water stress was conferred by greater allocation to roots. This was only considered to of benefit in ecosystems where winter precipitation results in the filling of the soil water profile at the start of the growing season. The most important trait identified was that of higher leaf turnover – the ability to shed leaves at the onset of water stress and regrow new leaves rapidly following rainfall clearly reduced the impact of severe water stress. This trait is associated with the same set of "fast-growth" traits that was related to defoliation tolerance in **chapter 5**. Therefore some association between responses to water stress and grazing is evident for C_4 grasses, with species more tolerant of water stress predicted to be more tolerant of defoliation as well.

Conclusion

The research included in the dissertation offers new insights into the determinants of ANPP in grasslands. While almost all studies of the effect of precipitation on ANPP have focused on annual precipitation, these results indicate that more accurate prediction of ANPP will require taking into account potential effects of intra-seasonal variation in precipitation, grazing and grass community composition. The effect of intra-seasonal variation was found to vary between sites, while the potential effect of species composition was reduced by non-selective grazing. In a future of changing precipitation patterns, ever-increasing threats to biodiversity and increased grazing of rangelands to meet the demands of growing human consumption, these factors can only become more important as determinants of grassland ANPP. While the subset of C_4 grass species studied revealed a large diversity of responses to precipitation and grazing amongst co-occurring grass species, the use of functional groups and predictive traits provides a potential means to extrapolate the results to many more species.