Grass Root Gaps: an Establishment Bottleneck Limits Trees in Savannas and Excludes them in South Africa's Highveld Grasslands

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Abstract:

The Highveld grasslands of South Africa are climatically capable of supporting trees, yet no trees occur. The fact that no fire adapted savanna trees occur in these grasslands confounds the suggestion that fires maintain these ecosystems. This study addresses the question of what prevents savanna trees from surviving. It was hypothesized that grass root competition for space eliminates the availability of suitable 'gaps' for tree establishment under high rainfall conditions.

Demographic bottlenecks on *Acacia* species populations were determined by examining the ratio of juvenile plants to adult carrying capacity. Below-ground root biomass was quantified in 10cm layers to a depth of 40cm at six sites along an elevation and rainfall gradient. The $\delta 12C/\delta 13C$ ratio was determined for roots collected. High rainfall sites were limited at the tree seedling establishment phase. Fine root biomass to 40cm depth increased with rainfall from 613 g/m^3 (Standard deviation = 235.1) to about 1326 g/m^3 (Standard deviation = 573.1). The surface layer had significantly higher fine root biomass than other layers, with site mean biomass in the surface layer increasing with rainfall ($p<0.00001$, $F_{3, 423}= 18.621$). Available gaps for tree establishment decreased with increasing rainfall/elevation; no gaps were found at the Highveld grassland site.

The findings of this study point to a strong savanna tree demographic bottleneck exerted, at the seedling establishment phase, by below ground grass competition for space. The Highveld grasslands receive to high rainfall for grass roots gaps to occur and permit seedling establishment.

Introduction:

South Africa's Highveld is characterized by fire dependent C_4 grasslands (Acocks 1953). Forest patches and exotic tree plantations found in these systems suggest that they have the capacity to support a much larger woody biomass (O'Connor and Bredenkamp 1997). This study aims to address the 'savanna-grassland problem' (Mills *et al.* 2006), namely the puzzles of tree exclusion in grasslands and tree-grass co-existence in savanna systems. This 'problem' is not limited to Southern African ecosystems, and a multitude of studies have taken place on similar grassland systems around the world (Wakeling *et al.* 2010), in addition to the vast body of research on global savanna systems (Sankaran *et al.* 2004).

Bond *et al.* (2005) concluded that fire is an important factor preventing global potential vegetation (predicted by climate) from being achieved. The fire regime of South Africa's Highveld grasslands was suggested by Bond *et al.* (2003a) as the factor preventing trees from growing there. Using a Dynamic Global Vegetation Model, their study showed that the high rainfall grasslands became dominated by woody growth forms in the simulated absence of fire. This does not, however, account for the inability of fire-adapted savanna trees to thrive in grasslands (Wakeling *et al.* 2010). O'Connor and Bredenkamp (1997) reviewed other hypotheses, including frost sensitivity of trees and the poor drainage of Highveld soils as factors excluding trees. In both cases, exceptions such as frost resistant trees, and grasslands occurring on well-drained soils render these hypotheses limited (Mills *et al.* 2006). Wakeling *et al.* (2010) hypothesized that tree seedling growth is reduced by lower nutrient availability in the Highveld soils, and that this growth reduction prevents tree seedlings from escaping the fire trap, to explain the absence of trees. Although growth was constrained in grassland soils, these authors concluded that the observed difference in growth between savanna and grassland soils was not a general factor limiting trees.

The possibility that grass competition is more intense in Highveld grasslands than in savannas, and causes the absence of trees was suggested by Mills *et al.* (2006). Walter (1971)'s root niche separation theory, which states that trees and grasses are able to co-exist by occupying and obtaining resources from different soil layers, is confounded by the fact that in some cases savanna tree roots do occupy the top layer of soil (Sankaran *et al.* 2004) (e.g. Mordelet *et al.* 1997 in humid savannas and February and Higgins (2010) in mesic savanna), and the fact that savanna trees cannot avoid occupying the same portion of the soil as grasses during the seedling establishment phase (Jurena and Archer 2003). Indeed, experimental studies have shown intense competitive effects of grass on tree seedling recruitment in this system (Kraaij and Ward 2006, Cramer *et al.* 2007, Cramer *et al.* 2010), and larger size classes (Riginos 2009). Grasses can be assumed to be better competitors for below ground resources (February and

Higgins 2010), and quicker responders to improved growth conditions. Furthermore, recent work has suggested that grass root competition can alter tree seedling root architecture and growth (Messier *et al.* 2009).

Demographic models offer multi-factorial explanations of tree limitation in savannas and grasslands. Higgins *et al.* (2000) proposed a model where savanna tree seedling establishment (hereafter 'establishment') is limited by rainfall, and recruitment from sapling to adult phase (hereafter 'recruitment') is controlled by fire. Thus, establishment occurs erratically during periods of increased rainfall; this is clearly not supported by high rainfall grasslands such as the Highveld, which does not exhibit the anticipated 'storage effect' implied by the model. Like the study by Wakeling *et al.* (2010), this study aims to build upon the demographic framework outlined by Higgins *et al.* (2000). As most grass competition is below ground, and possibly more intense in grasslands than savanna (Mills *et al.* 2006), an establishment bottleneck is potentially maintained by competition for space below ground (Bond 2008). Jurena and Archer (2003) demonstrated the importance of available gap area (i.e. space below ground free of grass roots), particularly in the vertical plane, on tree seedling establishment. Grass root biomass was also shown to vary a great deal spatially and temporally in the *Prosopis* savanna in which the study was conducted, with considerably higher below ground biomass during the higher rainfall year (Jurena and Archer 2003). It was concluded that the savanna site studied had sufficiently large below ground gaps to allow seedlings to establish; this study aims to investigate the suggestion put forward by Bond (2008) that very high root biomass in South Africa's Highveld grasslands may create an establishment bottleneck for savanna trees.

To ascertain whether demographic bottlenecks are in fact present in savanna tree populations, and if present, at which phase of the tree life-cycle they are acting, I determined the ratio of juvenile trees to an adult carrying capacity. This was carried out at C_4 grass systems along a gradient of increasing elevation and rainfall to accommodate savanna and grassland sites in the kwa-Zulu Natal province of South Africa. It is predicted that under high rainfall conditions, tree density is constrained by an establishment bottleneck (reflected in low juvenile: carrying capacity ratio), while under intermediate rainfall conditions, fire and/or herbivory create recruitment bottlenecks (reflected in a high juvenile: carrying capacity ratio). Grasses are predicted to be better competitors for resources below ground, and it is expected that their root dominate the soil profile. It is also predicted that soils are capable of supporting trees, and no nutrient bottlenecks occur throughout the elevation/rainfall gradient, as per the findings of Wakeling *et al.* (2010). I quantified grass root biomass at six sites along the elevation/rainfall gradient to investigate 'gaps' for tree establishment to take place. A 'gap' was quantified in terms of a threshold biomass value below which establishment could occur. In line with Jurena and Archer

(2003)'s findings that higher rainfall resulted in greater root biomass, higher rainfall grassland sites are anticipated to have higher grass root biomass, (especially in the surface layer), and fewer, if any, 'gaps'.

It is hoped that the outcomes of this study contribute to the disequilibrium-based theory of tree-grass co-existence (Scholes and Archer 1997, Sankaran *et al.* 2004), through a demographic perspective of tree populations. In doing so, it is hoped a better understanding of tree exclusion in grasslands and tree limitation in savannas may be brought about, with the eventual goal of empowering management of these systems in the face of contemporary challenges such as conservation, climate change, bush encroachment, and permutations thereof.

Study Site:

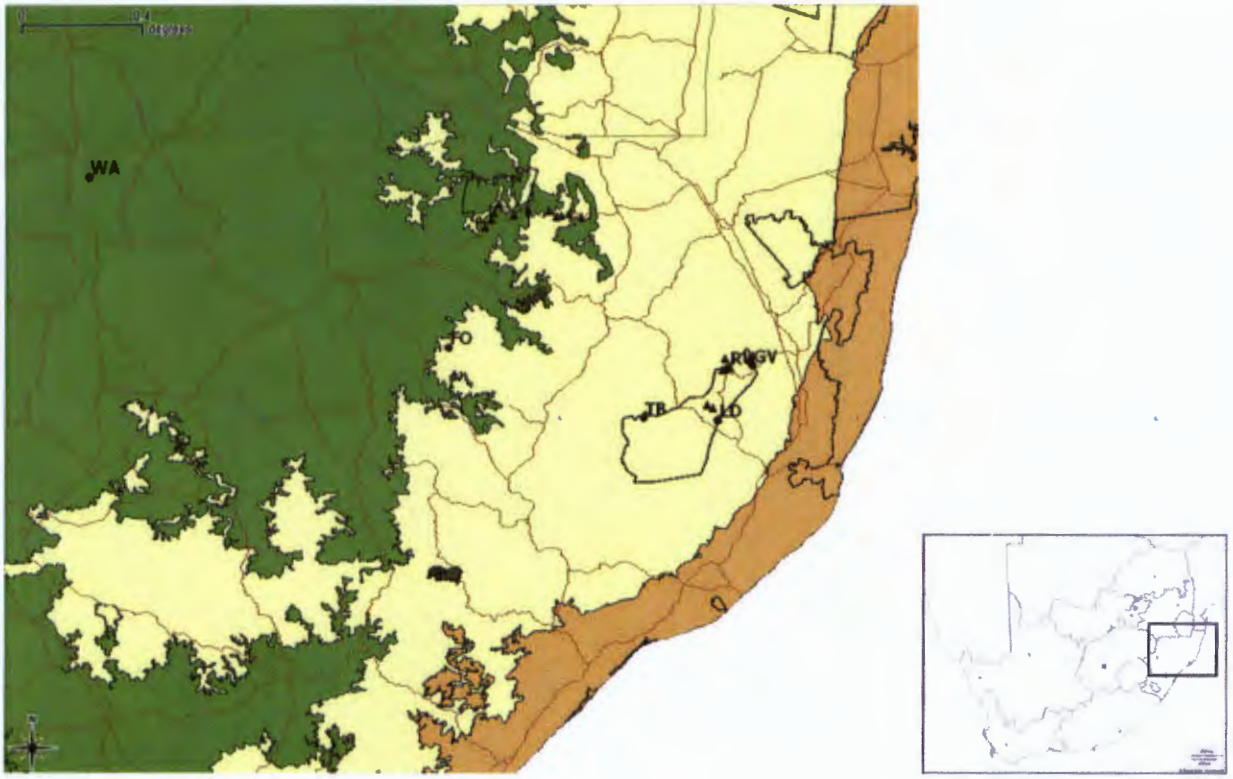

Figure 1: Map of portion of East coast of South Africa, showing root excavation sites (circles) (see Table 1) and transect sites added to dataset of Staver *et al.* (2009) (triangles). National Park boundaries outlined in black, provincial boundaries outlined in grey. Roads shown in brown. Biomes: yellow= savanna; light green= grassland; dark green= forest; brown= Indian Ocean Coastal Belt.

Materials and Methods:

Seedling-Establishment Bottleneck Analysis

I censused *Acacia* (see Appendix for species) tree densities in different size classes at a large number of sites in kwa-Zulu Natal province of South Africa to determine whether there was any trend in recruitment limitation. Sites were chosen to extend across rainfall and tree density gradients; site selection was constrained by accessibility from the road.

Adult tree carrying capacity was determined in a site- and species-specific manner by calculating the maximum number of tree canopy areas able to fit in a 1 ha area. The average canopy diameter of a tree species at a particular site was used in calculating canopy area. Those sites without measured canopy diameters were assigned the value K= 250 trees/ha (close to the K obtained using the overall average canopy diameter from all the sites: 199 trees/ha). Size classes were defined as juvenile (<1m height), medium (<1.5m height), and adult (>1.5m height), and censuses were performed across 2 x (125m x 2m) transects. A recruitment index, n/K (where n= number of small+medium plants/ha; K= carrying capacity of adult trees/ ha), was calculated for each site.

A recruitment index was obtained, in an identical fashion to above, for sites from a database collected by Staver *et al.* (2009). This database contained *Acacia* height class information collected in 40m x 10m plots extensively covering lower elevations in HiP, as well as fire frequency, dung counts, and elevation of plots.

Site Rainfall

For sites within HiP, site elevation was converted to a Mean Annual Precipitation (MAP) value following Balfour and Howison (2001), who derived the following:

Rainfall = 1.164*(altitude) + 462 (R^2 = 0.94).

Sites outside HiP were plotted as a GIS layer on top of the BioClim 12 layer in Manifold, and MAP values read off from co-ordinates.

Accounting for Nutrients:

Soil samples

The barrel auger (head diameter= 7.2cm, length= 14cm) was used to take a soil core to 40cm from a location at the centre of the sampling grid. Each 10cm layer was extracted separately, then sieved, and

roots removed in the process. Sub-samples of the 10cm and 40cm samples were sent to BemLab Laboratories (Strand, Western Cape) for analysis of pH, Electrolytic Conductivity (EC), Total Phosphorous (Total P), Bray II P, Na, K, Ca, Mg, %C, %N, NO_3, NH_4, T-value, and texture and soil type classification.

'Gap' Analysis:

Six sites with a range of tree densities were selected along an elevation/rainfall gradient, including two grassland sites with no trees, in the kwa-Zulu Natal Province of South Africa. Functional, near natural disturbance regimes (fire, herbivory, or both) were a pre-requisite for sites falling outside the boundaries of Hluhluwe-iMfolozi Park, leading to the selection of two private commercial farm sites in the vicinity of Wakkerstroom and Gluckstadt. The two grassland sites were WA and RU, site RU occurring as a grassland bounded by scarp forest and Zululand Lowveld.

Table 1: Study site summary of experiment quantifying above and below ground biomass at sites along an elevation/rainfall gradient. HiP= Hluhluwe-iMfolozi Park. Vegetation classification from Mucina *et al.* (2005).

Site	Code	Location	Vegetation	Elevation (amsl)
Thoboti	TB	HiP (iMfolozi)	Zululand Lowveld	195
Le Dube	LD	HiP (Hluhluwe)	Northern Zululand Sourveld	290
Gontshi Viewpoint	GV	HiP (Hluhluwe)	Zululand Lowveld	178
Rubbish Dump	RU	HiP (Hluhluwe)	Zululand Lowveld+ Scarp Forest	484
Ford Farm	FO	Vicinity of Gluckstadt	Income Grassland/Northern Zululand Sourveld	816
Wakkerstroom	WA	Vicinity of Volksrust	Amersfoort Highveld Clay Grassland	1644

Above Ground Heterogeneity

Field work took place from November 2009-March 2010. Using calibrated 2m poles, a 6m x 3m grid was constructed in an open, adult tree-free site. Disc Pasture Meter (DPM) readings were taken at 1m intervals along the grid, starting at the point (0.5m; 0.5m); two more rows starting at (1.5m; 0.5m) and (2.5m;0.5m) were also measured, resulting in three rows per site. The centre of the area measured by the DPM was marked using fluorescent-paint covered toothpicks to create 18 such marked points per site. At each point, the % vegetation cover within the area falling under the DPM-measuerd zone was estimated. The distance from the marked point to the nearest grass bunch was measured using a tape measure; the grass was identified, and the tape measure was used to measure the bunch diameter. Two more species of grass occurring in the immediate surrounds were identified (if occurring).

Below Ground Heterogeneity

At each of the 18 marked grid points, a steel barrel auger (head diameter= 7.2cm, length= 14cm) wa used to extract soil and roots in successive 10cm intervals up to a depth of 40cm. Where it was impossible to reach a depth of 40cm due to rock being encountered, an adjacent site was selected. The soil and root contents of each layer were stored separately in brown paper bags.

The 72 bags per site were transported to the Zululand Tree Project (ZLTP) base at Hluhluwe-iMfolozi Park. Here, ZLTP staff extracted all roots from the soil by hand. Roots from each sample were then placed in small buckets and flushed with water. Once floating on the surface, roots and organic matter were removed separately using a fine sieve (~1mm).

Following washing, root samples were dried in an oven at 40°C for three days. Once dry, weighing took place with large (>2mm diameter) and small (<2mm diameter) roots from each sample weighed separately.

'Gap' quantification

In the absence of data regarding lateral root expansion and root architecture, for example, gaps in the grass root biomass that permit the establishment of tree seedling were defined as follows: those points at which the fine root biomass to a depth of 40cm< lower quartile (25 percentile) of all point biomass to 40cm across all sites.

Root isotope signal:

$\delta^{13}C$ analysis was performed on root samples to determine the relative contributions of C_4 grasses and trees (C_3) to the biomass collected. Dried, weighed roots were transported to University of Cape Town Botany Department. Root samples from points of a randomly selected row at each site were ground to a fine particle particle size using an ONESTO KC10-T In D16 grinder with 1mm sieve. Where sample sizes were very small, a Retsch (model MM200) ball bearing grinder was used until a fine powder state was achieved. Care was taken not to handle samples and to clean equipment (using compressed air for the ONESTO grinder and ethanol for the Retsch mill after each grinding to avoid contamination. Only fine roots (<2mm diameter) were analysed for site FO; coarse and fine roots were analysed at all other sites.

About 1mg of samples from a single row randomly selected at each site was weighed to 1 microgram on a Sartorius micro-balance into tin cups; tin cups were then squashed to enclose the sample. Combustion of samples took place in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). Gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Bremen, Germany). $\delta^{13}C$ values obtained were corrected using Sucrose (from Australian National University), and dried *Nasturtium* leaf standards. These standards have been calibrated against International Atomic Energy Agency standards. Carbon values obtained are relative to Pee-Dee Belemnite.

Data Analysis:

Root weights were converted to g/m^3 values and then log transformed (base 10, c=1) to meet assumptions of normal distribution. Total Root Biomass of each site's grid points (i.e. combined root biomass from each depth for all 18 points per site) were compared with a single-way ANOVA using the program STATISTICA (StatSoft 2009). A depth-wise comparison was also performed using a single-way ANOVA in STATISTICA (StatSoft 2009) on the combined values of all the sites for each depth interval. For both ANOVA's, Tukey HSD post-hoc tests were performed to identify where differences occurred.

A Principal Components Analysis was undertaken in STATISTICA (StatSoft 2009) on soil characteristics in surface (top 10cm) and 30cm-40cm layers.

In assessing the relationship between above and below ground biomass, Multiple Regression analysis was performed using STATISTICA (StatSoft 2009)) to determine the relationship between above ground

biomass, grid point-tussock distances, and tussock diameters, and below ground biomass to 40cm, and in the top 10cm.

Community Analysis Program (CAP) was used to assess similarity of grass species assemblages of the sites; specifically, DECORANA Ordination was used.

Results:

Demographic Bottleneck Analysis:

Sites with MAP from ca. 680mm to ca. 800mm had the highest occurrence of n/K > 1 and were less limited by seedling establishment. At lower rainfall sites there was greater variability in n/K values, and more sites are seedling limited. Sites with MAP exceeding 800mm were the most seedling limited sites of all, having the lowest n/K values (Figure 2).

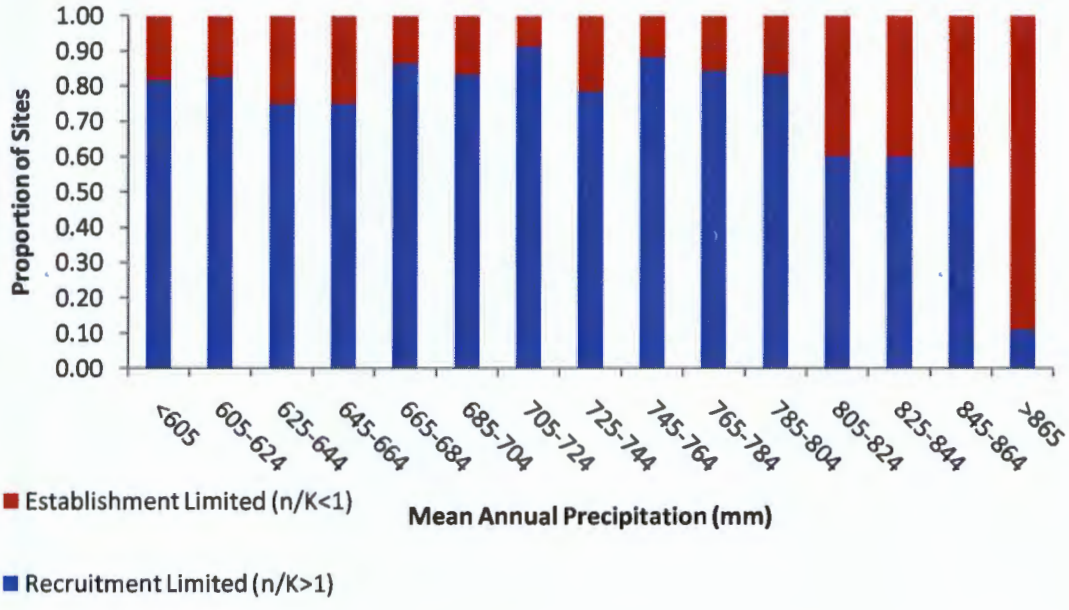

Figure 2: Summary of population bottlenecks of *Acacia* spp. along a rainfall gradient. Includes savanna sites from Staver *et al.* (2009) and grassland sites. 'n' refers to juvenile density per hectare, 'K' is adult tree carrying capacity per hectare. K derived as 1 ha/ mean canopy area of adult tree. n/K>1 indicates site tree density is limited by recruitment from sapling to adult ('recruitment' bottleneck), n/K<1 indicates site tree density is limited by tree seedling establishment ('establishment' bottleneck). See Appendix Table 1 for table summary.

Surprisingly, sites in HiP subject to very high fire return intervals or very high herbivory intensity were not seeding establishment limited, the majority having n/K values > 1. Thus, neither herbivory intensity nor fire frequency appear to influence seedling establishment (Figure 3 (a) and (b) respectively).

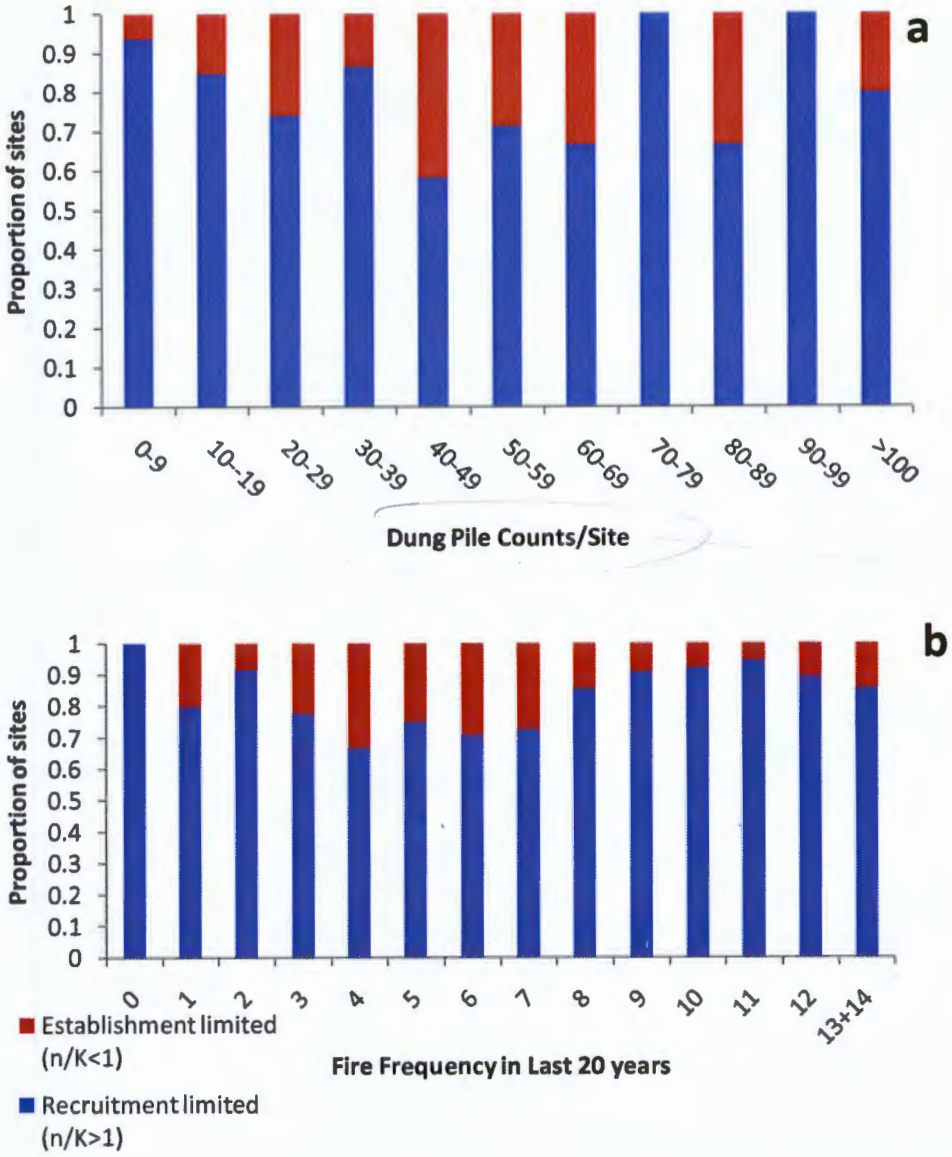

Figure 3: Influence of **a)** herbivory intensity (indicated by dung pile counts) and **b)** fire frequency over last 20 years on adult tree density of *Acacia* spp. in Hluhluwe-iMfolozi Park. Data from Staver *et al.* (2009). n/K>1 indicate site tree density is limited by recruitment from sapling to adult ('recruitment' bottleneck), n/K<1 indicates site tree density is limited by tree seedling establishment ('establishment' bottleneck).

Soil Analysis

In both the surface (10cm) and 40cm layers of soil, pH and Total Cations follow very similar trends. pH and Total Cations decrease with elevation in both layers, although the trend is stronger in the 40cm layer (see Appendix, Figure 1). The high elevation sites (RU, FO and WA) have lower N content (%N, NH4-N and NO-N than the lower elevation sites in the surface layer in particular. The Bray 11 P correlation with elevation in the surface layer may be a product of very high values for sites WA and FO which is possibly due to fertilizing (see Appendix, Tables 3 and 4).

Root Isotope Signal:

Although C_3 signals appear in throughout the rainfall gradient, the lower rainfall sites have a greater proportion of spikes in $\delta^{13}C‰$ (Figure 5) across all depths. Site FO is the only site that does not provide a C_3 signal in roots collected. There is a weak pattern of increasingly negative $\delta^{13}C$ values with increasing depth.

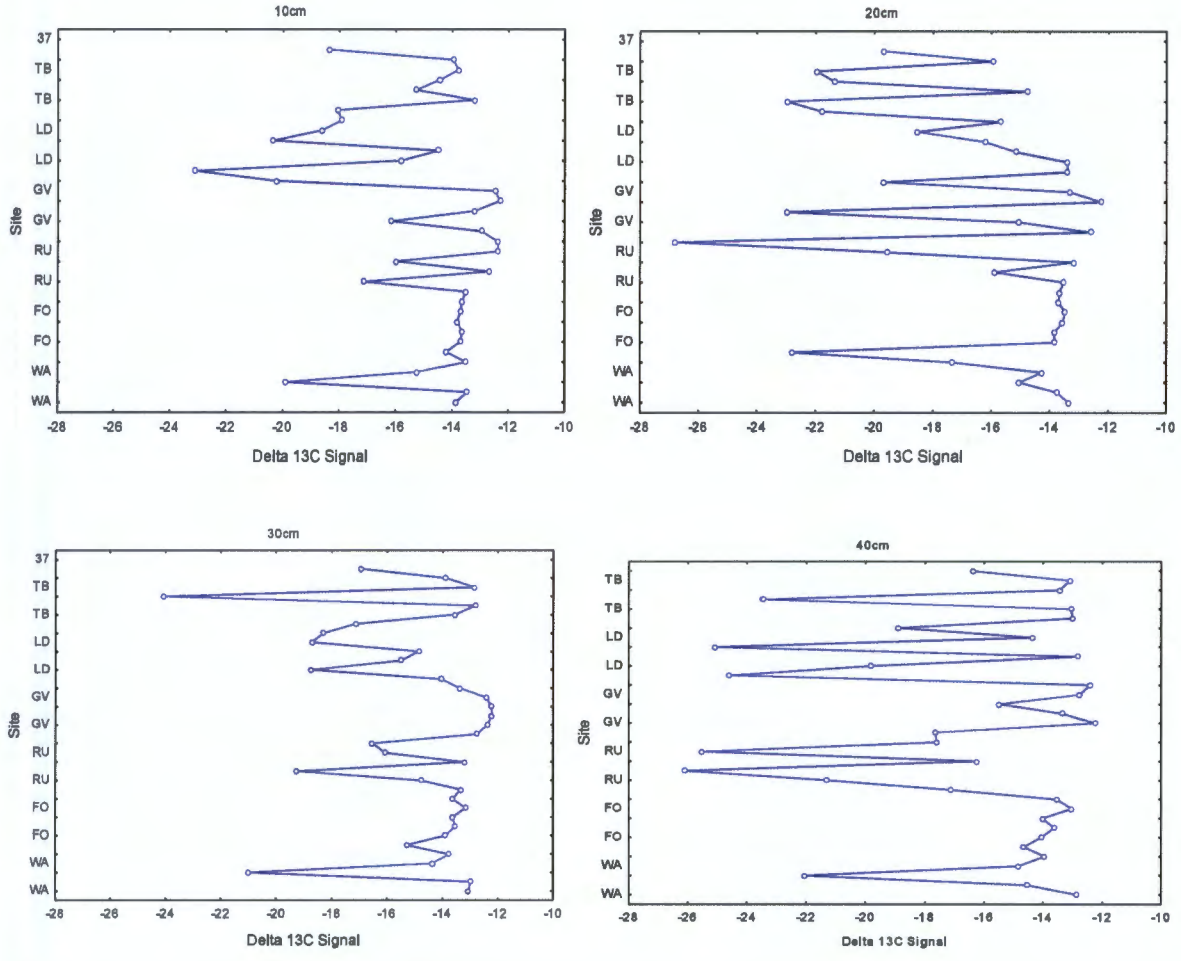

Figure 4: Standard corrected $\delta^{13}C‰$ (relative to Pee Dee Belemnite) of all roots in each depth from six points along one row at each site along an elevation/rainfall gradient. Highveld grassland: WA, grassland (Hluhluwe-iMfolozi): R; savanna (Hluhluwe-iMfolozi): TB, LD, GV; savanna: FO.

Heading?

Point fine root biomass to a depth of 40cm ranged from approximately 343 g/m^3 (site TB) to approximately 3051 g/m^3 (site WA). Mean fine root biomass to a depth of 40cm ranged from about 613 g/m^3 (Standard deviation = 235.1) (site LD) to about 1326 g/m^3 (Standard deviation = 573.1) (site WA) (Figure 5). The sites differed statistically in their mean fine root biomass to a depth of 40cm ($p<0.00001$, $F_{5, 102}= 18.051$); a summary of site differences is found in Table 2. The lower rainfall sites (TB and LD) had comparable mean biomass to 40cm, while intermediate rainfall sites (GV and RU) and high rainfall sites (FO and WA) followed a similar pattern; sites RU and FO overlapped into high and intermediate rainfall groups respectively.

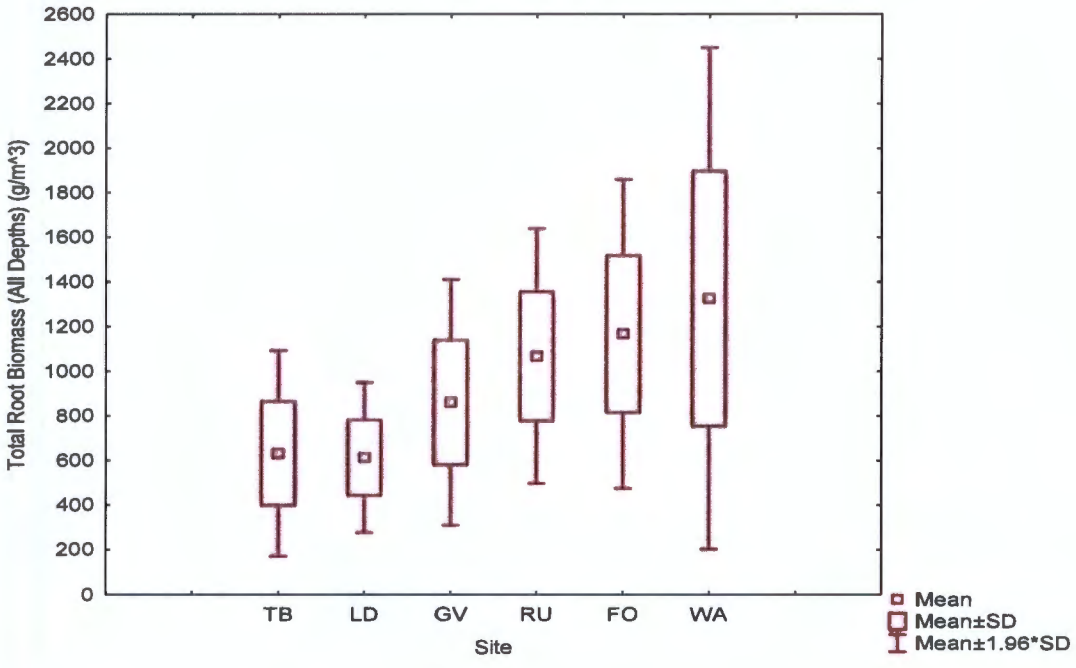

Figure 5: Box and Whisker summary of fine root biomass to a depth of 40cm at 18 grid points in each of six sites along a l-r increasing elevation/rainfall gradient. Highveld grassland site: WA, grassland site: RU, savanna sites: TB, LD, GV, FO.

Table 2: Summary of Tukey HSD post-hoc test of ANOVA (performed in STATISTICA) on fine root biomass to a depth of 40cm at six sites along a l-r rainfall gradient. Values are approximate probabilities, * denotes significant difference. n each site = 18, df = 102.

	TB	LD	GV	RU	FO	WA
TB		1.0000	0.0354*	0.0001*	0.0001*	0.0001*
LD	1.0000		0.0327*	0.0001*	0.0001*	0.0001*
GV	0.0354*	0.0327*		0.2674	0.0517	0.0028*
RU	0.0001*	00001*	0.2674		0.9769	0.5374
FO	0.0001*	0.0001*	0.0517	0.9769		0.9293
WA	0.0001*	0.0001*	0.0028*	0.5374	0.9293	

Fine root biomass within a depth interval declined exponentially as depth increased ($y = 3748e^{-0.0632x}$, R^2 = -0.89, $p<0.5$) (Figure 6). Across all the sites, the surface layer (top 10cm) had the highest biomass (mean = 2223 g/m^3), and was the most variable (standard deviation = 1329) of the depth intervals (Figure 4).

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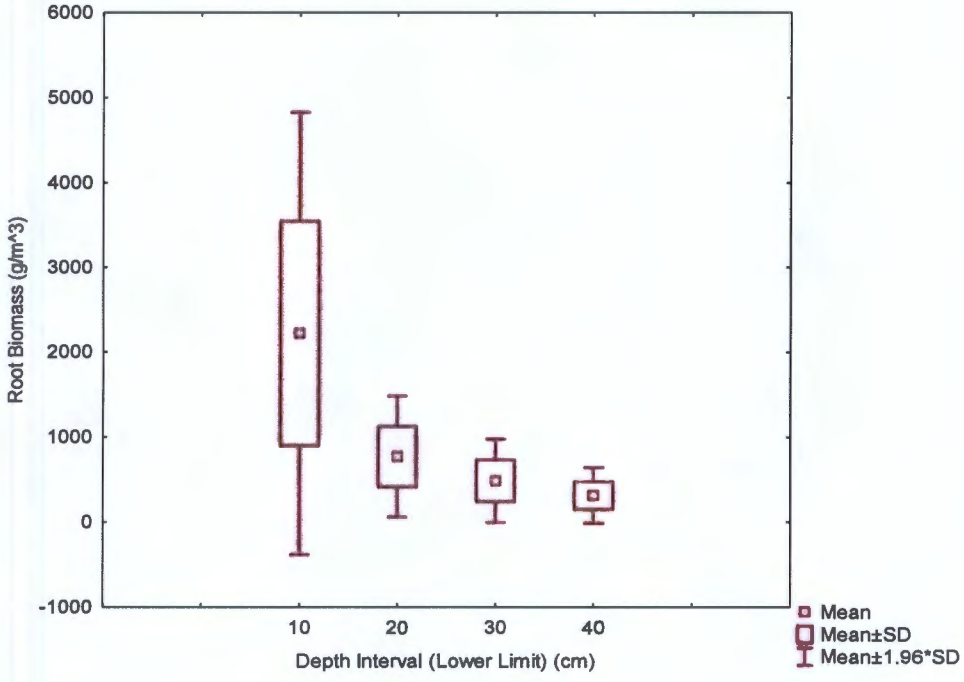

Figure 6: Box and whisker summary of fine (<2mm diameter) root biomass per depth interval collected from six sites (two grassland, four savanna) along an elevation/rainfall gradient. n for each depth interval = 108.

A similar pattern to the total point fine root biomass to 40cm is observed in each of the depth intervals individually. Generally, the higher rainfall sites have higher fine root biomass; (Figure 7) shows the highveld grassland site WA having the highest mean fine root biomass in the 10cm and 20cm intervals. This trend is most pronounced in the top 10cm depth interval, where sites along the rainfall gradient differed significantly in terms of fine root biomass ($p<0.00001$, $F_{3, 423}= 18.621$). A Tukey HSD post-hoc test showed that at the 10cm depth interval, sites grouped exactly as for total fine root biomass to 40cm (i.e. according to higher, intermediate and lower rainfall groups with sites RU, FO overlapping between intermediate and higher groups) (error between MS= 0.055, df= 102). Differences between sites decreased with depth (Figures 6 and 7), and at 30cm and 40cm depth intervals, only site FO differed statistically from sites LD and GV respectively (error between MS= 0.510, df= 102).

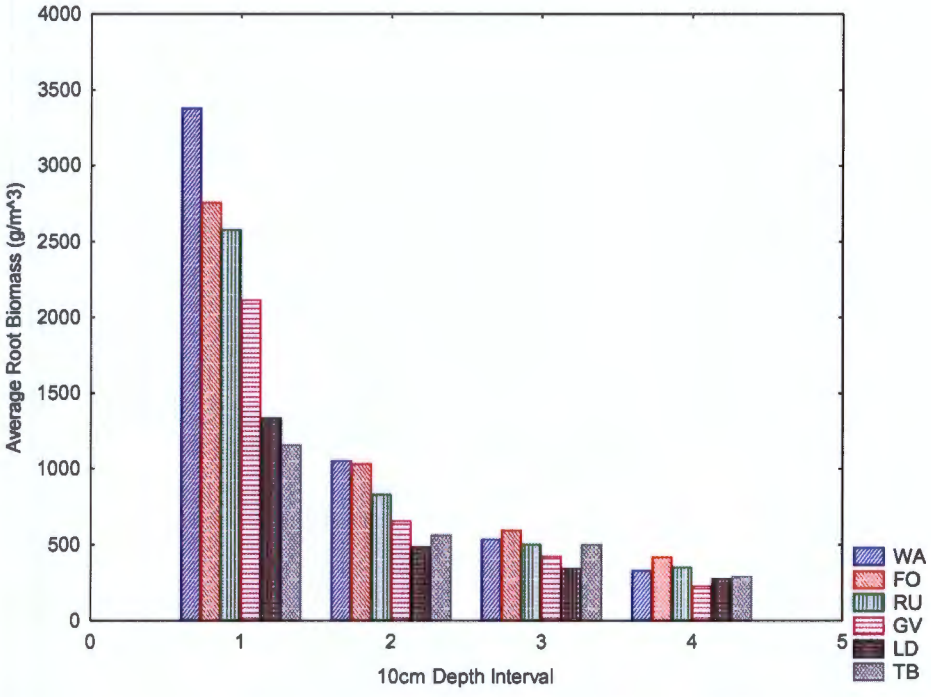

Figure 7: Average fine root (<2mm diameter) biomass for all four 10cm depth intervals at each of the six sites situated along a savanna-grassland continuum. Sites arranged in descending rainfall/elevation order. **10cm** mean: 2223 g/m^3 std. dev.: 1329; **20cm** mean: 771 g/m^3 std. dev.: 362; **30cm** mean: 448 g/m^3 std. dev.: 250; **40cm** mean: 315 g/m^3 std. dev.: 166. Highveld grassland: WA, grassland (Hluhluwe-iMfolozi): R; savanna (Hluhluwe-iMfolozi): TB, LD, GV; savanna: FO.

Elevation and rainfall are strong predictors of grass communities' distribution, as the six sites form distinct entities in terms of grass species composition and abundance according to this gradient (Figure 8). Sites LD and GV are the most similar, while TB and WA are the least similar. TB (arid savanna) is characterized by the species *Digitaria argyrograpta* and *Panicum maximum*, LD by *Cymbopogon excavatus*, GV by *Digitaria eriantha* and *Hyperthelia dissolute* in particular, RU by *Tristachya leucothrix*, FO by *Melinis repens*, and WA by *Eragrostis chloromelas*..

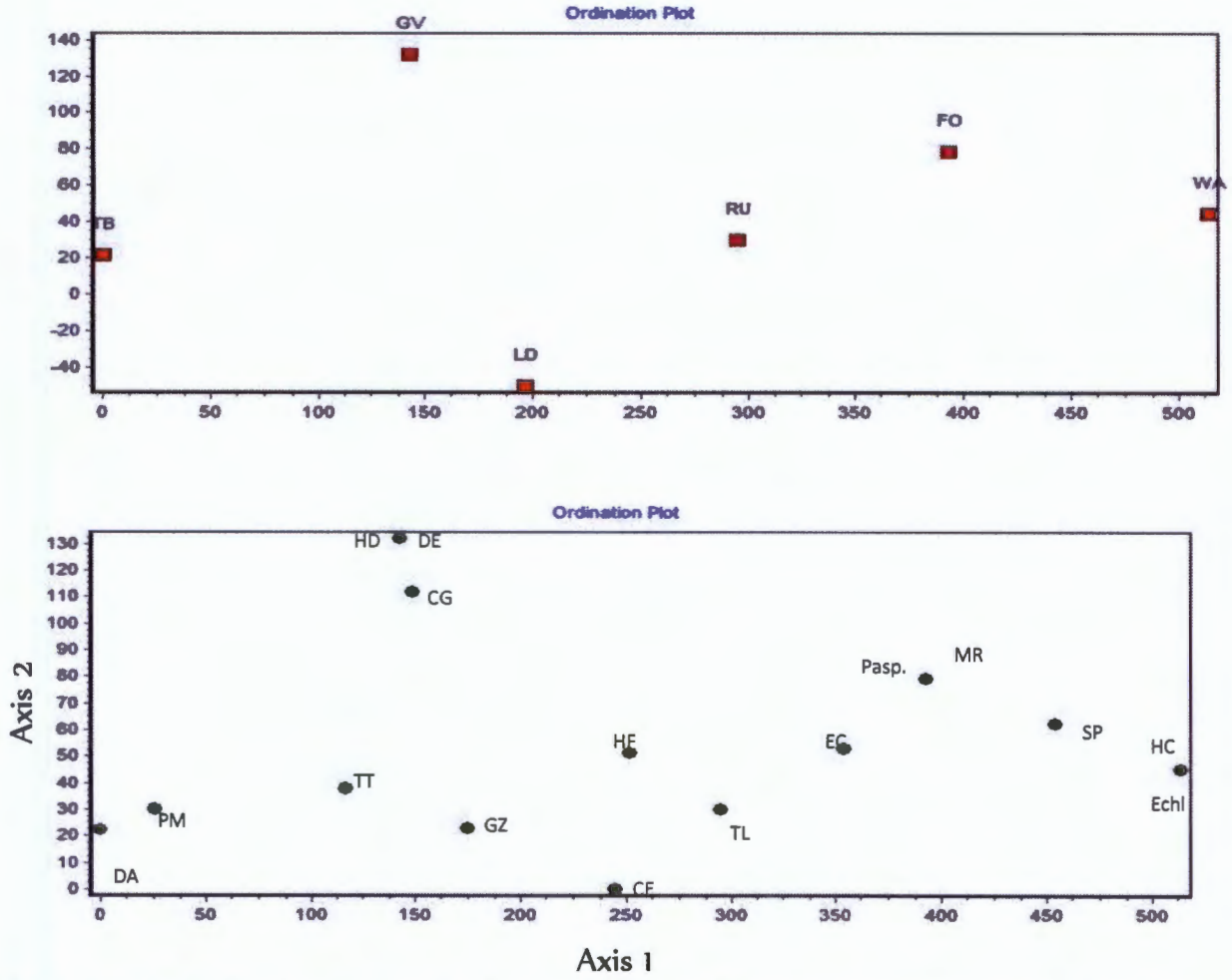

Figure 8: CAP Ordination showing distribution of sites (top) by species composition and abundance (bottom). Sites situated along elevation/rainfall gradient. Eigen values: Axis 1: 0.9295, Axis 2: 0.1248. Highveld grassland: WA; grassland (Hluhluwe-iMfolozi): RU; savanna (Hluhluwe-iMfolozi): TB, LD, GV; savanna: FO. See Appendix (Table 2) for species-code conversion.

The number of root gaps suitable for seedling establishment decline as rainfall and below ground biomass to a depth of 40cm increase (Figure 9). No root gaps exist at sites FO and WA, the highest rainfall sites, while the most gaps occur at LD. Higher spatial heterogeneity is found at the high rainfall end of the gradient (Figures 5 and 9).

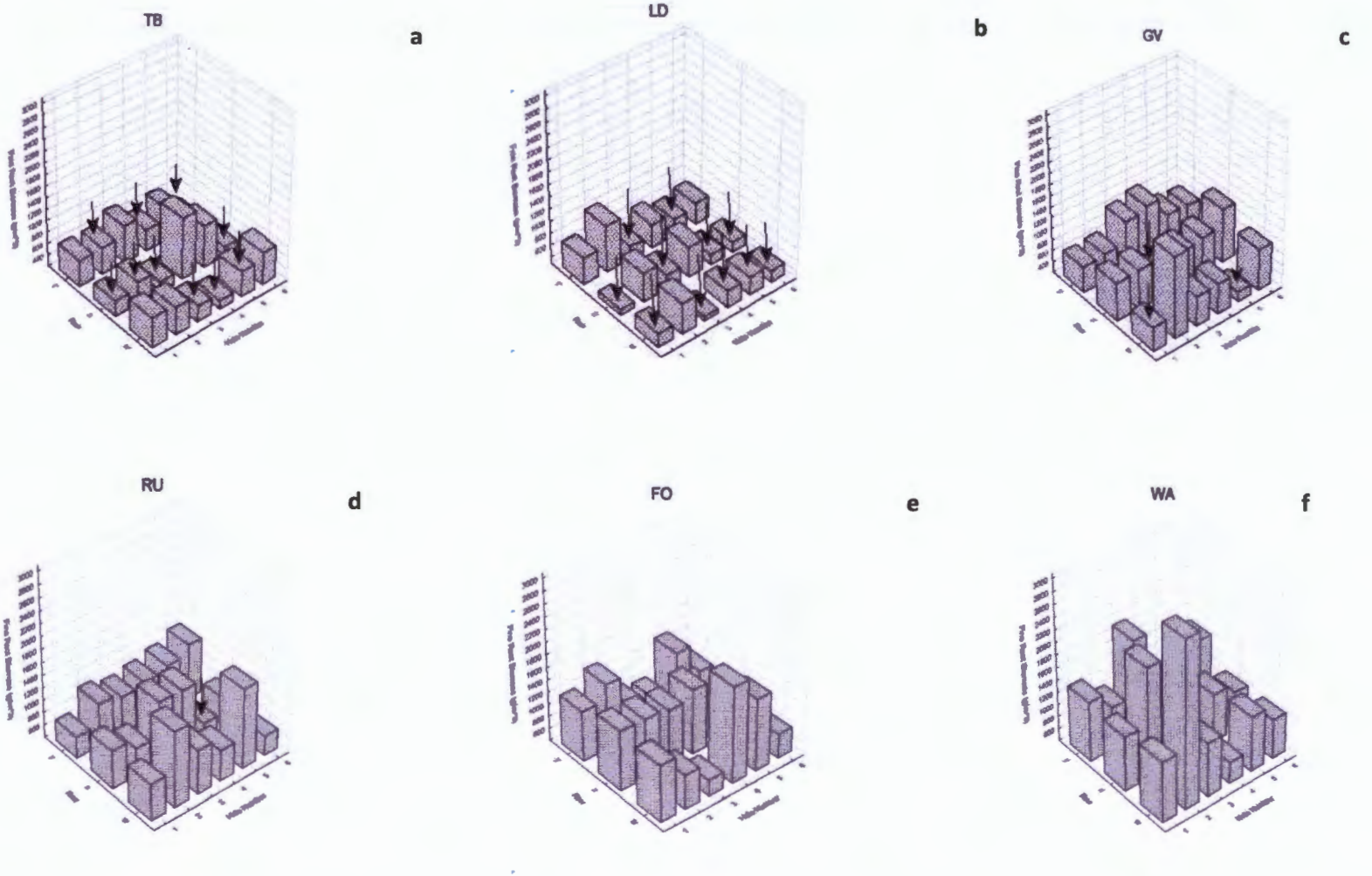

Figure 9: Mean fine root biomass to a depth of 40cm in six sites along an increasing (a-f) rainfall gradient, showing tree seedling establishment gaps (black arrows). Gaps defined as points with fine root biomass< 657g/m^3 (lower quartile of point fine root biomass to 40cm across all sites combined). TB- mean: 632 g/m^3 std. dev.: 235; LD- mean: 613 g/m^3 std. dev.: 171; GV- mean: 860 g/m^3 std. dev. 281; RU- mean: 1068 g/m^3 std. dev. 290; FO- mean: 1167 g/m^3 std. dev.: 353; WA- mean: 1326 g/m^3 std. dev: 573. Axis Labels: X: 'Hole Number' Y: 'Row' Z: 'Fine Root Biomass (g/m^3)'. Highveld grassland: WA, grassland (Hluhluwe-iMfolozi): RU; savanna (Hluhluwe-iMfolozi): TB, LD, GV; savanna: FO.

Above ground biomass correlated with neither total fine root biomass to 40cm, nor fine root biomass in the surface (top 10cm) layer at any of the sites, separately or combined; the strongest relationship observed was at site RU where a weak relationship was observed ($R^2 = 0.332$, $p = 0.01229$, $df = 1, 16$, $m =$ positive) between total fine root biomass to 40cm and above ground biomass emerged. The portion of grid sites falling onto grass bunches did not correlate with rainfall, average distance of grid points to the nearest bunch, diameter of nearest bunch, or point above ground biomass.

Discussion:

Higgins *et al.* (2000) assumed that rainfall limited seedling recruitment in semi-arid savanna but not in mesic savanna. Erratic rainfall has also been explained as limiting tree populations in arid savannas of Namibia and the Kalahari (Wiegand *et al.* 2005). The recruitment index used here (n/K) does show more sites with recruitment limitation (n/K<1) at lower rainfall sites. Contrary to Higgins *et al.* (2000)'s assumptions, establishment limitation increased at higher rainfall sites. At least for the HiP sites, establishment was not strongly influenced by herbivory nor fire (Figure 3 (a) and (b)). This is consistent with Staver *et al.* (2009) who found no altered trend in establishment in a long term exclosure experiment. Midgley and Bond (2001) have pointed out that size-class distributions may be misleading in their utility as indicators of demographic bottlenecks, as short *Acacia* plants could be newly established or older resprouts recovering from fire or herbivory. This is not a huge concern within the realm of this study in light of the lack of influence herbivory and fire have been shown to have in the HiP system.

pH and Total Cations of soils respond similarly, throughout the profile, to soils sampled by Wakeling *et al.* (2010) along a similar elevation/rainfall gradient. The Highveld grasslands occur on ancient, leached soils that have fewer nutrients than those at lower elevation (King 1978, Partridge 1997). My results show that pH (and Total Cations) decrease with elevation (Figure 1 in Appendix, Tables 3 and 4 in Appendix). Wakeling *et al.* (2010) concluded that pH in was a good indicator of soil fertility for *Acacia karroo* and *Acacia sieberiana*; my results therefore confirm the trend observed in their study of grassland soils being less suitable for *Acacia* savanna trees than savanna soils. An assumption of this study is that although soils sampled at my sites show a decreasing suitability to tree growth with increasing elevation and rainfall, even at the top end of this gradient it is not biologically significant enough to completely exclude savanna trees, as concluded by Wakeling *et al.* (2010).

The majority of roots collected were C_4 roots and therefore of graminoid origin (Figure 4). There were no trees present in the Highveld grassland site (WA), and the low $\delta^{13}C$ point from this site is a forb

signal. Using this as a control, it appears that tree roots do occur in the top 10cm alongside grass roots, as well as at greater depths, confirming that no separation occurs between grass and tree roots, as found by February and Higgins (2010) and Mordelet *et al.* (1997). More C_4 plants occur in the lower rainfall sites as expected under lower grass competition. Ideally, the signal of fine root biomass (<2mm) would be presented, to confirm that those roots used to quantify below ground biomass are in fact of graminoid origin. This is the case for site FO, where large roots were excluded. The procedure of extracting roots used here is very prone to contamination, throwing some uncertainty on these findings in general.

The establishment limitations at high rainfall therefore are not imposed by herbivoy, fire, or nutrients. Increased establishment limitation is, however, consistent with more competition from grasses. The root excavation studies show increasing fine root biomass, especially in the surface layer, with increasing rainfall so that savanna and grasslands differ in mean root biomass. However, my prediction related to spatial heterogeneity (below ground gaps) decreasing from savanna to grassland. Establishing criteria for a root gap proved to be a challenge with the data collected. Jurena and Archer (2003) used specific length and diameters to create exact volumes of 'gaps' by using PVC tubes to exclude grass roots. Unfortunately I was unable to find a means of equating below ground biomass to 'available volume' from the study of Jurena and Archer (2003), inhibiting the use of the 10cm diameter threshold elucidated by these authors. To accommodate their finding that available space in the vertical plane was most important, I employed the lowest quartile of point fine root biomass to 40cm of all grid points at all the sites as a threshold of a root gap suitable for seedling establishment. Measured in this way, the proportion of points at each site being gaps suitable for tree seedling establishment (i.e. below the threshold biomass value) varied greatly amongst the sites (Figure 9). The most gaps were in the semi-arid savannas (sites LD and TB), while the Highveld grassland site (WA) had the fewest. Assuming dispersal is not a limitation, these sites present a decreasing number of establishment events possible as elevation and rainfall increase, until establishment is impossible. A question does arise as to how a high altitude site (FO) supports savanna when, according to my measure, no suitable gaps occur (at least not in the $18m^2$ sampled).

The high altitude savanna is characterized by *Acacia sieberiana*, while *Acacia karroo, Acacia nilotica, Acacia tortilis, Acacia robusta*, and several others frequent the lowlands (personal observation). Different *Acacia* species may have vastly different life history strategies to overcome various demographic bottlenecks (Midgley and Bond 2001). *Acacia sieberiana*, able to grow under lower nutrient conditions than lowland *Acacia* spp., possibly has a higher tolerance to the presence of grass roots than the lowland species. Messier *et al.* (2009) demonstrated that late successional tree seedlings

do not exhibit root segregation responses to grass competition, while early successional trees do, to the detriment of seedling growth. Perhaps a combination of low nutrients, high rainfall, and fire and herbivory have resulted in adaptations to interference for space below ground in *Acacia sieberiana*, while lowland species are adapted to grow roots into space following disturbances that generate mortality in grasses (e.g. fire (Bond and Midgley 2001)). The implications of this are that different tree species likely have different gap requirements, with the smaller root gaps in the *Acacia sieberiana* savanna (site FO) possibly being masked by the crude gap measure. Under even higher rainfall, such as at site WA, it is proposed that grass root biomass is above root gap thresholds of all trees.

The huge variability in fine root biomass in the top 10cm across all sites compares with the spatial variability observed by Jurena and Archer (2003);, a surprising result is the variability of fine root biomass to a depth of 40cm in the higher rainfall sites (FO and WA). It was predicted that lower rainfall sites would have the greater heterogeneity, and as a result, more root gaps. However, the vast majority of the point biomass values to 40cm at these sites are still above the root gap threshold. As Jurena and Archer (2003) note, spatial heterogeneity probably reflects the fact that below-bunch biomass can be twice as much as between-bunch biomass, as concluded by Brown and Archer (1989). The high rainfall received at these sites may result in more grass bunches establishing, and hence, greater below ground heterogeneity. From personal observations, this holds true in the Highveld grassland site (WA), where dominant grass species are *Eragrostis chloromelas* and *Heteropogon contortus*, which are smaller growth forms than, for example, *Cymbopogon excavatus*, (prominent at LD) (Figure 8), and therefore occur in higher density.

Successful establishment in root gaps is compounded by above ground gap requirements; space is needed for resources such as light. The lack of correlation between below ground fine root biomass and above ground variables such as biomass once again mirror the findings of Jurena and Archer (2003); the conclusion in their case is that high above and below ground variability create a multitude of micro-sites that could enable tree establishment. This could apply to the low-intermediate rainfall sites in this study, where larger graminoid forms and high proportions of root gaps conspire to create this effect. Ultimately, the lack of an obvious relationship between above ground variables and below ground fine root biomass prevents the easy assessment of the suitability of grassy stands to tree establishment (Jurena and Archer 2003).

Future research and comments:

The high variability of site n/K values at the lower end of the rainfall gradient could be a similar signal to that observed by Wiegand *et al.* (2005), where sites have received a range of rainfall amount in recent

seasons, and tree seedlings have established accordingly. It would be interesting to confirm whether the findings of Wiegand and Ward do apply to the semi-arid to mesic savannas dealt with here; an examination of the n/K status of sites with a consideration of the recent rainfall record would be an obvious starting point, and expectations are that sites with MAP< 600mm have higher seedling establishment during normal and high rainfall seasons, and lower establishment during low rainfall years. It follows that Figure 1 potentially shows the effects of high rainfall in the preceding season(s). Alternatively, mesic sites could be grass competition limited instead, having sufficient rainfall and limited by available 'gaps' for establishment.

Improvements can be made to the gap quantification methods employed here. Ideally, a similar technique to that employed by Jurena and Archer (2003) would be used, where a variety of diameter/length variations of several different volumes could test the survival of tree seedlings at the sites used in this study. Of interest would be whether different *Acacia* tree species have different root gap size requirements for establishment. Depending on whether roots overlap to a large degree with grass roots in the upper layers of the soil, the importance of space in the vertical plane is expected to change. To this end, and for the benefit of the whole study, another site at the boundary between grasslands and savanna may have provided more insight.

The influence of the time of year that the root excavations took place must be considered. Root biomass was quantified in the middle of the growing season, and probably represents a near maximum. Of interest is the status of grass competition at the end of the dry season; it is expected that the same patterns would emerge, but biomass would be lower, and in savanna sites there may be a window of more root gaps for tree establishment.

Insights into the question of what limits trees in grasslands and savannas around the globe may be taken from this finding. Incorporating the effects of grass competition for space below ground into spatially explicit models of savanna patch dynamism is a particularly useful application of the results of this study (Jurena and Archer 2003). For example, the model generated by Meyer *et al.* (2007) (SATCHMO) could incorporate grass root competition for space into the below ground competition component to model savanna succession at sites with rainfall higher than 650mm, to provide insight into patch scale dynamics. From the results of the model, landscape-scale predictions of sensitivity to woody encroachment for example may be derived, and manage altered accordingly (Meyer *et al.* 2007).

Conclusions:

The findings of this study point to a strong savanna tree demographic bottleneck exerted, at the seedling establishment phase, by below ground grass competition for space. Under higher rainfall conditions on nutrient-poorer soils, grass root biomass increased in quantity and heterogeneity, and suitable root gaps for the establishment of trees decreased until non-existant in the Highveld grassland and savanna sites. Thus, availability of root gaps is a strong limiter of tree density in mesic savannas, and excluder of trees in the Highveld grassland of South Africa.

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Appendix:

Abbreviations:

DPM= Disc Pasture Meter

HiP= Hluhluwe-iMfolozi Park

MAP= Mean Annual Precipitation

ZLTP= Zululand Tree Project

Table 1: Summary of Figure 1 showing proportions of sites establishment limited (n/K> 1) in rainfall intervals. n= number of juvenile trees, K= maximum density of adult trees/ha derived from canopy diameter data.

MAP (mm)	No. Sites	Sites	Prop. n/K>1
<605	11	hlu,imf	0.82
605-624	23	hlu,imf	0.83
625-644	20	hlu,imf	0.75
645-664	12	hlu,imf	0.75
665-684	22	hlu,imf	0.86
685-704	24	hlu,imf	0.83
705-724	23	hlu,imf	0.91
725-744	14	hlu,imf	0.79
745-764	17	hlu,imf	0.88
765-784	19	hlu,imf	0.84
785-804	6	hlu,imf	0.83
805-824	15	hlu,imf,out	0.60
825-844	5	hlu,imf,out	0.60
845-864	7	hlu,imf,out	0.57
>865	9	hlu, out.	0.11

Tree Species Analysed in Transects (including Staver *et al.* 2009):

Acacia borlei

Acacia burgessii

Acacia burkei

Acacia gerrardii

Acacia grandicornuta

Acacia karroo

Acacia nigrescens

Acacia nilotica

Acacia robusta

Acacia sieberiana

Acacia tortilis

Table 2: Names and codes of grass species used in a CAP Ordination (DECORANA plot) in Figure 7. Grass species found at six sites (two grassland, four savanna) along an elevation/rainfall gradient.

Code	Species
CE	*Cymbopogon excavatus*
CG	*Chloris gayana*
DA	*Digitaria argyrograpta*
DE	*Digitaria eriantha*
E. chl	*Eragrostis chloromelas*
EC	*Eragrostis curvula*
GZ	*Setaria sphacelata*
HC	*Heteropogon contortus*
HD	*Hyperthelia dissoluta*
HF	*Hyparrhenia filipendula*
HH	*Hyparrhenia hirta*
MR	*Melinis repens*
PD	*Panicum deustum*
PM	*Panicum maximum*
SP	*Sporobolus pyramidalis*
TL	*Tristachya leucothrix*
TT	*Themeda triandra*

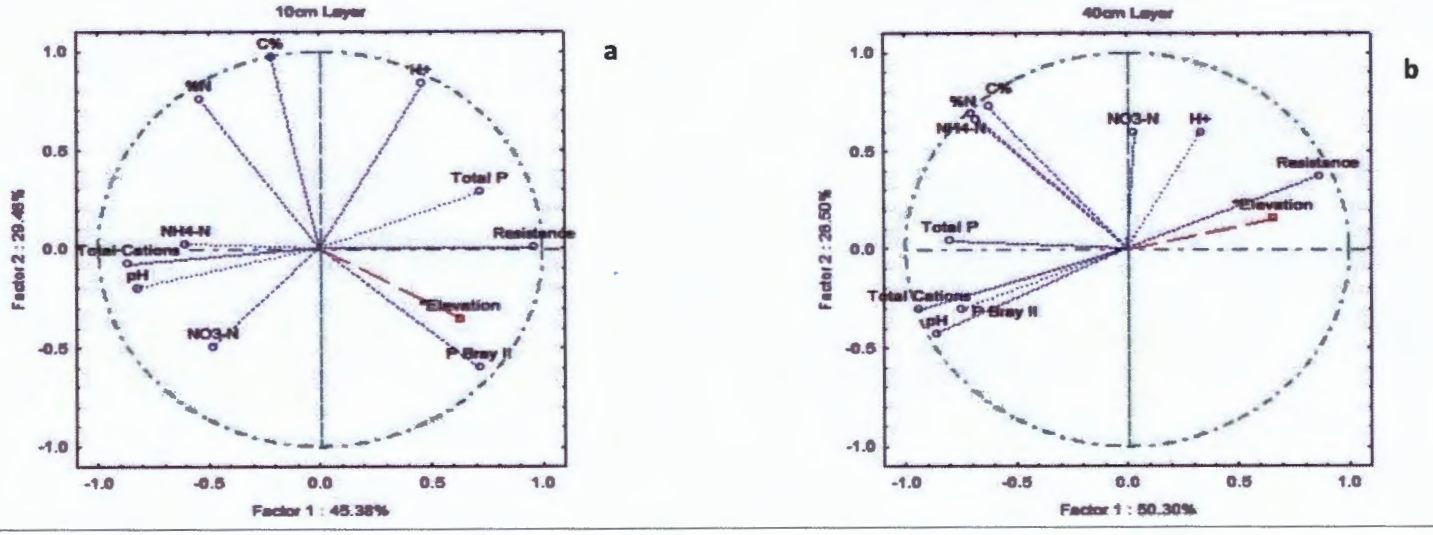

Figure 3: STATISTICA (StatSoft 2009) Principal Component Analysis (PCA) of soil variables from samples taken from six sites at (a) 10cm and (b) 40cm layers. Sites situated along elevation gradient, which is a supplementary factor in the analysis. Highveld grassland: WA, grassland (Hluhluwe-iMfolozi): R; savanna (Hluhluwe-iMfolozi): TB, LD, GV; savanna: FO.

Table 3: Summary of 30cm-40cm soil layer characteristics. Data displayed in Figure 3 (a) in Appendix

Site	Elevation (m amsl)	pH	Resistance	H+	P Bray II	Total Cations	C%	Total P	%N	NO3-N	NH4-N
WA	1644	4.3	2340	1.23	5	3.38	1.72	168.68	0.115	36.08	68
FO	816	4.8	2810	0.94	10	5.93	1.67	193.96	0.125	34.16	57.44
RU	484	4.5	2120	2.61	1	8.12	3.95	198.24	0.24	34	61.6
GV	330	5.4	990	0.79	1	16.95	2.52	128.87	0.192	34.8	66.48
LD	290	5.3	1470	0.94	2	14.55	3.34	166.47	0.275	35.6	68.8
TB	195	5.5	860	0.59	3	22.72	2.02	175.68	0.184	35.92	62.48

Table 4: Summary of 30cm-40cm soil layer characteristics. Data displayed in Figure 3 (b) in Appendix

Site	Elevation (m amsl)	pH	Resistance	H+	P Bray II	Total Cations	C%	Total P	%N	NO3-N	NH4-N
WA	1644	4.3	3210	0.99	2	2.62	0.93	146.25	0.106	36	58.16
FO	816	4.8	2220	0.84	1	7.54	1.14	132.24	0.073	35.04	63.92
RU	484	4.4	2590	2.51	2	7.42	2.92	164.95	0.234	34	62.72
GV	330	5.4	1140	0.94	4	23.92	4.9	227.2	0.422	35.92	79.2
LD	290	5	1720	1.08	0	12.45	2.73	92.69	0.179	34.56	62.88
TB	195	5.6	830	0.44	5	25.64	1.08	201.23	0.114	33.52	57.92