

Aboveground vs. belowground biomass investment of seedlings might explain floristic composition across a tropical forest-savanna boundary



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Universiteit Utrecht



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Abstract

In the seasonal tropics, a dynamic balance can exist between forest advance and retreat. Forest retreat is caused by fire, but forests can advance into savannas in the absence of fire. The present study focuses on limitation of forest expansion in the absence of fire. This is tested by gathering transect data and conducting a nursery experiment. The floristic composition changes gradually from mature forest to open savanna. Forest seedling establishment is critical in understanding the dynamics of forest advance. This establishment is constrained by drought, low soil nutrient availability, competition with herbaceous vegetation, and defoliation by fire and herbivory. This study shows that in the absence of fire, patches of closed savanna exist, but forest species are not dominating. Forest species advance is strongly and negatively correlated with herbaceous cover while it is strongly and positively correlated with canopy cover and soil nutrients. Additionally, comparative growth analysis on forest and savanna seedlings shows that the impact of grasses is stronger on forest species than on savanna species. This could be explained by different ecological trade-offs that savanna and forest seedlings make to adapt to their respective environments. Forest species allocate more in above ground biomass than savanna species, which might allow them to compete for light in forest. However, this makes them bad competitors for belowground resources compared to savanna seedlings that invest more in belowground biomass. These results indicate that in the Soutpansberg, South Africa, forest advance into savannas might only be possible if canopy closure by savanna species alters the environment favorable for forest seedling establishment.

Introduction

Much of the seasonal tropics are structured in a mosaic of forest and savanna. This can be either on a landscape or a regional scale (Longman & Jenik, 1992). Savannas are defined as: “a community having a continuous grass layer scattered with trees” and forest as: “consisting predominantly of woody plants and from which grasses are virtually absent” (Adejuwon & Adesina, 1992). The savanna-forest boundary represents the natural limit to the distribution of these two vegetation types. Understanding factors that determine the location of this boundary helps to predict vegetation distribution in a changing climate and disturbance through land use. This is especially true for the savanna-forest boundary. Isotope analysis showed that savannas now exist where forest existed before (Desjardins et al., 1996). Biome switches, either forest changing to savanna, or savanna changing into forests have consequences for biodiversity and ecosystem functioning (Bond, 2008). For example, in Brazil large areas of Amazon forest changed into savannas due to human disturbance (Cochrane & Laurance, 2002). Forest expansion into savannas is a form of bush encroachment. In South Africa alone, thirteen million hectares are already bush encroached severely disrupting ecosystem functioning and the capacity to support livestock (Kraaij & Ward, 2006).

At the savanna-forest boundary there is a dynamic balance in forest retreat and forest advance. In forest retreat, fire is seen as the major factor (Bond, Midgley, & Woodward, 2003). Due to intolerance of forest species to fire (Hoffmann, Orthen, & Nascimento, 2003), frequent savanna fires degrade the boundary and cause mortality among forest species. Therefore, increasing fire frequency leads to large amounts of forest turning into savanna. In the absence of fire, forest can advance into savannas (Hopkins, 1992). Fire exclusion experiments in Gabon showed that trees rapidly grew there (King, Moutsinga, & Doufoulon, 1997). Contrary to these results, studies in Australia (Bowman & Fensham, 1992) and the Brazilian Cerrado (San José & Fariñas, 1991) revealed that decades of fire exclusion led only to a scattered amount of forest trees. This inconsistency of results shows that the process of forest advance is still not understood and empirical research is needed. Therefore, forest advance into savannas is the primary focus of this study.

An intrinsic feature of savanna-forest boundaries is that savanna and forest do not only differ in tree density, but also in species composition. Most forest tree species predominantly exist in forests and most savanna tree species are confined to savanna (Adejuwon & Adesina, 1992; Felfili & Junior, 1992). Differences in physiological and ecological factors between savanna and forests are expected to determine the distribution of these species (Longman & Jenik, 1992). Forest is often found on sites with greater water and nutrient availability, and savannas exist on dryer sites, where nutrient availability is lower (Furley, 1992). Further, in savannas trees have to compete with the herbaceous layer for these scarce resources, which can severely impact growth (Riginos, 2009; Scholes & Archer, 1997; Van der Waal et al., 2009). However, herbaceous cover has also been proved to facilitate seedling establishment (Anthelme & Michalet, 2009). Last, disturbances such as herbivory and fires are frequent in savannas (Van Langevelde et al., 2003). Light is abundant in savannas, but due to a

closed canopy cover in forest, light is the primary limiting resource for which competition exists (Frost et al., 1986; Grime, 1977).

One can postulate that the successful establishment of forest tree seedlings in savannas will determine the rate of forest expansion into savannas and is therefore crucial in understanding forest advance (Rossatto, Hoffmann, & Franco, 2009). Without seed germination and seedling establishment, tree abundance can not increase. We expect that this will be constrained by drought, nutrient availability, and competition with grass biomass (Knoop & Walker, 1985; Van der Waal et al., 2009). Further, during the initial life stages seedlings are vulnerable for browsing and fire (Scholes & Archer, 1997). It is however uncertain if communities composed entirely of savanna species can attain a forest physiognomy, or if forest seedlings invade before complete canopy closure (Rossatto et al., 2009).

To test if establishment of forest seedlings is constrained by savanna environments one needs to test if forest seedlings are more sensitive than savanna seedlings to savanna environmental factors. Phylogenetic independent comparisons between forest and savanna seedlings showed that they are both differently adapted to their respective environments. Hoffman et al., (2004) showed that forest seedlings had lower survival rates in savanna conditions than savanna seedlings. Further, nursery experiments revealed that savanna tree seedlings invested more in below ground resource capture and storage, and forest seedlings invested heavily in stems and leaves (Hoffmann & Franco, 2003). Also, forest seedlings had a higher specific leaf area (leaf area per leaf mass, SLA) and leaf area ratio (leaf area per total plant mass, LAR) than savanna seedlings. It is assumed that this enables savanna species to resprout after defoliation events such as fire and herbivory, and can better compete for belowground nutrients. Aboveground biomass investment allows forest species to compete for light in forests. Further, savanna seedlings exhibit greater plasticity in above described leaf traits (Hofmann & Franco, 2003). This might enable them to better cope with a more heterogeneous environment (Bazzaz, 1996). The capacity of a seedling to adapt its traits to different environments might influence its success in different vegetation types. However, only the effect of two different nutrient and light levels were tested and the role of competition with grasses has not been tested yet.

In the upper reaches of the Soutpansberg (South Africa), a mosaic of savanna-forest vegetation occurs. There are several vegetation types with a clear gradient in canopy and herbaceous cover, from mature evergreen forest to transitional thicket, and closed woodland to open savannas. Fire has been absent for at least fifteen years in the study sites, so this is an ideal location to study forest advance into savanna. To test if forest advance occurs I hypothesize that:

- *Forest trees are predominantly found in forest but not in savannas, whereas savanna species are dominating in savanna, but not in forest.*
- *In transitional vegetation types with an intermediate cover and grass layer, forest trees will dominate.*
- *Forest species domination is expected to positively correlate with canopy cover, nutrient and water availability, soil organic matter (SOM) and to negatively correlate with light availability, temperature and*

herbaceous cover. No negative correlation between herbaceous cover and forest species indicates forest advance.

These hypotheses were tested by gathering in situ data. Secondly, the following hypotheses were tested:

- *Total biomass production of savanna tree seedlings will be higher than forest tree seedlings under nutrient poor conditions and when competing with grass, whereas under shaded conditions forest species will have a higher total biomass production.*
- *Forest tree seedlings will allocate more biomass to stems and leaves and have a higher LAR and SLA than savanna tree seedlings, whereas savanna seedlings will allocate more biomass to root structure.*
- *Savanna species are expected to show greater plasticity than forest species, meaning that the difference in traits will be bigger when exposed to variable conditions.*

These hypotheses were tested with a nursery experiment using congeneric or confamilial species pairs which will ensure phylogenetic independence.

Material & Methods

Study Area

The study was done at Lajuma Mountain Retreat, in the upper reaches of the Soutpansberg, South Africa, during the wet season from November 2009 until April 2010. The climate is semi-arid with mean rainfall of 730 mm year⁻¹, but variations are considerable (Mourik et al., 2007). The vegetation consists of Soutpansberg Summit Sourveld, Soutpansberg Mountain Bushveld and Northern Mistbelt Forest (Mucina & Rutherford, 2006).

Transect observations

To test the first hypotheses six different vegetation types with a gradient in canopy from closed forest to open savanna were classified by a local expert. The first three of the six locations were from tall evergreen forest to low evergreen forest and finally, semi deciduous forest. The next three types were three savanna vegetation types, were from closed savanna to open savanna. These were chosen 500m away due to anthropogenic disturbance is the area (fig 1).

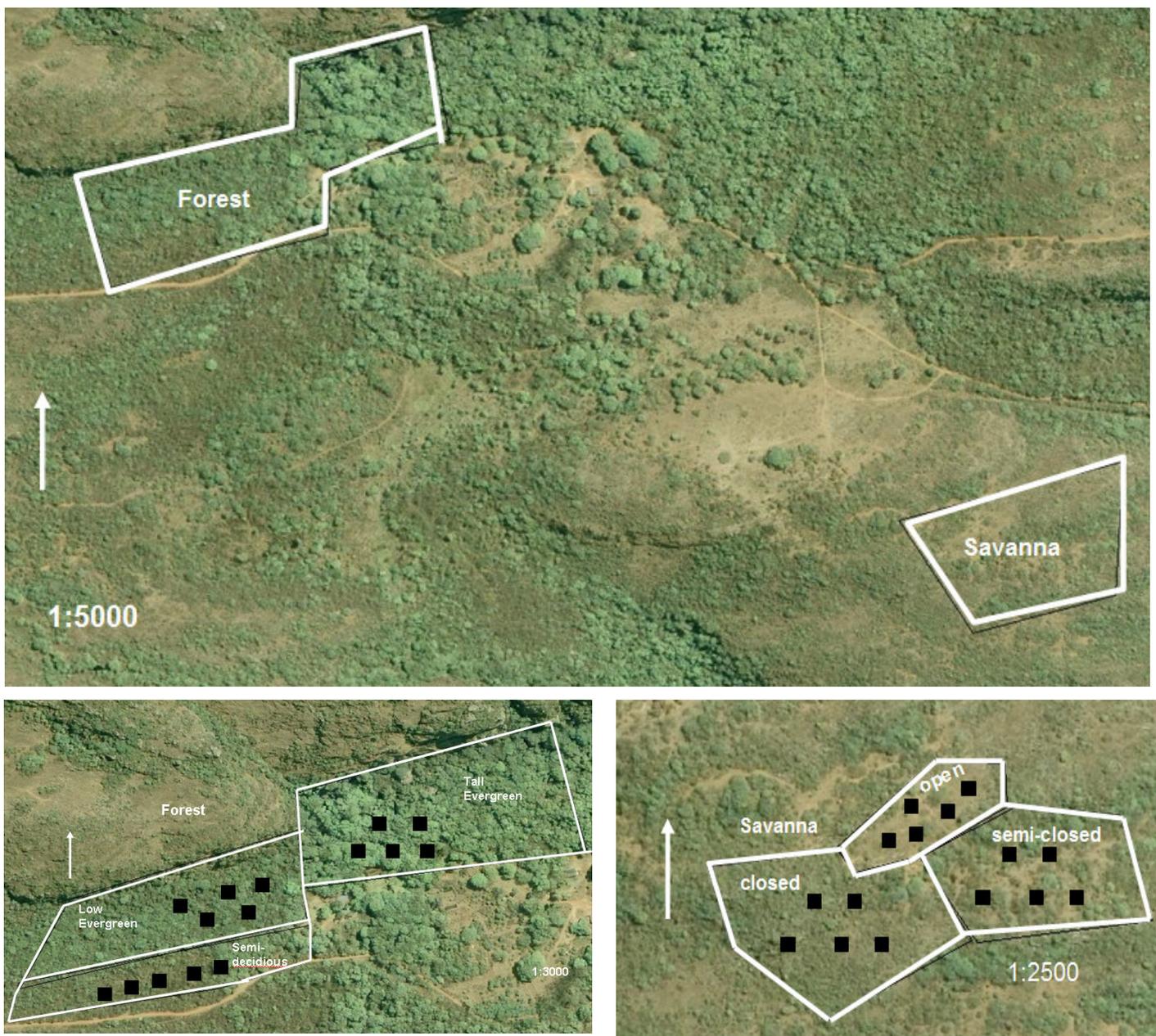


Figure 1 Overview of location of the two vegetation types (top), forest plots (left) and savanna plots (right). Satellite images of 2003. Plots were marked with GPS and image was created using ARCGIS. Black squares indicate the 5*5m plots. The 25*4m plots are not shown.

The forest vegetation types were officially classified as 'Northern Mistbelt Forest and the latter as 'Soutpansberg Mountain Bushveld' (Mucina & Rutherford, 2006). First, the vegetation boundaries were marked with a GPS. Then, systematically, five plots per vegetation type were chosen 20m apart, always facing south (fig 1). Per vegetation type, the five plots are assumed replicates. For the species data, plot size was 25*4m. This size was chosen so that sufficient trees were present in the plots. For the environmental variables plot size was 5*5m, in these plots was measured:

- Canopy cover (%)
- Canopy height (m)
- Herbaceous cover (%)
- Rock cover (%)
- Total amount of tree seedlings (<0,5m) and saplings (0,5-1m)
- Circumference at 1m, height, and canopy width of trees taller than 1m
- Soil depth

Soil samples of 0-30 cm were taken using an iron sampler. Nitrogen (N), phosphorus (P), soil moisture content and soil organic matter (SOM) were determined. All 30 samples were collected in one day between 7am and 2pm. The savanna plots were sampled before the forest plots to minimize variation in soil moisture content. Collection was after a week without rain, since dry conditions are most natural in the area. Soil moisture content was determined by weighing the fresh and dry weight after at least 48h drying at 100 °C. SOM was determined after drying for 3 hours at 500 °C. Trees were classified as forest, savanna, or general species using (Coate-Palgrave, 2002). Criteria were:

- Species occurring inside mature forest types and not in savanna vegetation types are forest species.
- Species not occurring in mature forest, and are occurring in open or closed woodlands, and only at forest margins are savanna species.
- Species occurring in a broad range of habitats, in both savanna vegetation types and inside forests are seen as generalist species.

All transect data was collected in January and February 2010, 2nd half of the rainy season. Analysis of variance was used to test for differences in the measured variables between vegetation types. Two tailed Pearson correlation was done to analyse correlations between mean amount of trees per species group and environmental variables. Ordination plots were made with Canoco 4.5 to visualize correlations between all environmental variables mean amount of trees per species group.

The nursery experiment

A nursery was constructed in an open area, and surrounding trees were cut to ensure similar shading. The roofing and walls were made with 20% shade cloth. Additionally, 80% shade cloth was used to construct a shade house (fig 2). Three forest and savanna congeneric or confamiliar species pairs, and three non relating species were chosen dependent on seed availability (table 1). Species were classified in two functional types, either forest or savanna, following the same criteria as explained in the transect section. Seeds were locally sourced and additionally ordered when needed from 'Silverhill Seeds South Africa'. Additionally, seeds were treated to instigate germination when necessary. Ten seeds were randomly chosen, dried for 24 hours at 70 °C and weighed.

Table 1: Species of two, savanna or forest, functional types grown in the nursery. Five savanna species and four forest species of which three congeneric or confamiliar species pairs and three additional species were chosen.

Savanna		Forest	
Family/Order	Species	Family/Order	Species
Myrtales, Combretaceae	<i>Combretum molle</i>	Myrtales, Combretaceae	<i>Combretum kraussi</i>
Myrtales, Myrtaceae	<i>Syzygium cordatum</i>	Myrtales, Myrtaceae	<i>Syzygium legatti</i>
Ericales, Sapotaceae	<i>Englerophytum megalismontanum</i>	Ericales, Sapotaceae	<i>Mimusops zeyheri</i>
Fabaceae, Papilionoideae	<i>Mundulea sericea</i>	Malpighiales, Euphorbiaceae	<i>Croton sylvaticus</i>
Fabaceae, Mimosoideae,	<i>Acacia karoo</i>		

Seeds were germinated on agar solutions or, with the larger Sapotacea seeds, in soil. Within three days after germination, tree seedlings were transferred to nursery bags of 20*20*50 cm. For each species, the initial weight of the original roots and stems were measured.

We applied the following treatments with ten replicas (fig 2):

- *Control = grass (-) / nutrients (-) & light (+)*
Sand is locally sourced which had a nutrient content of 1% P and 0.25% N.
- *Fertilizer*
45 g/m² Wonder 9:4:5 (N: 204.0, P: 114.6, K: 105.0 g/kg) was applied on the planting day and repeated every 4 weeks.
- *Grass competition*

After transplanting the germinated tree seedling into the nursery bag, substantial (+- 50) grass seeds of *Eragrostis curvula* were sowed homogenously in the nursery bag. *Eragrostis curvula* is a perennial and widespread grass in South Africa found in variable habitats (Zacharias, 1990).

- *Fertilizer & Grass competition*

Both the fertilizer and grass competition treatments were applied.

- *Shade/ Light (-)*

A shade house with 80% shade cloth was built to grow the tree seedlings.

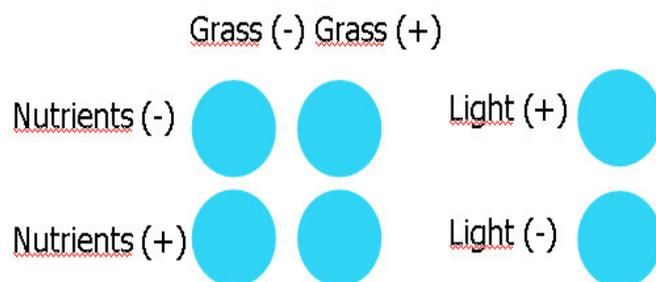


Figure 2: Two experimental designs of the nursery experiment (left) and the constructed nursery (right).

The blue circles represent different treatments. Nine species were studied, n=10 for all treatments. Note that the Grass (-)/Nutrients (-) is the same treatment as Light (+).

Due to time constraints and meagre seed availability, not a full factorial design was performed (fig 2). Since grass was expected to react on nutrients (Kraaij & Ward, 2006) a nutrient*grass factorial design was chosen, instead of nutrients*shade factorial design. Seedlings were placed randomly in the nursery. After planting, a plastic cup was placed on the seedling for one week, to maintain a moist environment. Almost daily, additional water was provided when needed to supplement natural rainfall. All treatments received the same amount of water. Sand chemical analysis (n=5), two weeks after fertilizer application, revealed that nutrient treatment contained 0.05% N and 0.02% P, which is double the amount of the control ($F_{3,16}=10.005$ $p<0.001$). Nutrient content was not different between the control, grass, and grass and nutrients treatments. PH did not differ significantly across all treatment ($p=0.42$).

After fourteen growth weeks destructive harvests were performed. Ten individuals per treatment were washed and the following measurements were done:

- Fresh and dry weight of stems, leaves, cotyledons, and roots.
- Root length and stem length.
- Leaf area and amount of leaves.

Leaf area was determined following Hoffmann & Franco, (2003). Three leaves per seedling were scanned on a flatbed scanner, and leaf area was determined using multiple image processor (MIP) plug-in of ImageJ. Dry weights were determined after drying for 24h at 70 °C.

Due to different fruiting times of trees seedlings did not grow similar amount of days, ranging between eleven and fourteen weeks. Species often show a short hyper exponential growth immediately after germination, followed by a long and slow decline in relative growth rate towards maturity (Hunt & Cornelissen, 1997). Conditions did not change during growth, since they all grew in summer. Therefore, I transformed the outcomes linearly to the amount of growth days, so comparison between species was possible. Unfertilized, grass had an average biomass of 75g*m² and when fertilized, an average of 950g*m² after 14 growth weeks.

Classical growth analysis was performed to determine growth and allocation patterns. Total seedling weight (TSW) was the total biomass at the harvest minus the starting weight. Biomass allocation patterns such as root mass fraction (RMF), leaf mass fraction (LMF), and stem mass fraction (SMF) were obtained by dividing the specific mass by TSW. Specific mass was the total biomass of the plant part minus the starting weight. The specific leaf area (SLA) and leaf area ratio (LAR) were obtained by dividing total leaf area by leaf weight (SLA) or total seedling weight (LAR). Analysis of variance was used to test the effects of treatments (fixed factors) on the measured traits (dependent variables). Outliers with more than 2.5 times standard deviation were removed. Additional, homogeneity of variance was tested and data was log transformed when necessary to meet the ANOVA assumptions (Zar, 1999). When testing for generalized differences between savanna and forest seedlings and for interactions, both the nutrient*grass*type and light*type design were tested (fig 2). When effect of treatment was significant post hoc Tukey tests were performed to test for differences between treatments.

Results

Transects

Forest plots were associated with a closed and high canopy, rocks, soil moist, and nutrients. Savanna plots associated with herbaceous cover (fig 3a). Forest species tree species was correlated to canopy cover ($r^2=0.678$, $p<0.001$, fig 4a) and negatively with herbaceous cover ($r^2=-0.672$, $p<0.001$, fig 4b). Similarly, savanna species were negatively correlated with canopy cover ($r^2=-0.671$, $p<0.001$, fig 4a), and positively with herbaceous cover ($r^2=0.689$, $p<0.001$, fig3b). Canopy cover was strongly negatively correlated with herbaceous cover ($r^2=-0.783$, $p<0.001$, fig3b). Further, canopy cover was positively correlated with soil N, soil P, soil moisture content, and SOM ($r^2=0.523$, $p=0.004$; $r^2=0.646$, $p<0.001$; $r^2=0.664$, $p<0.001$; $r^2=0.559$, $p=0.002$, fig 3b). Herbaceous cover was negatively correlated with the same variables ($r^2=-0.369$, $p=0.049$; $r^2=-0.518$, $p=0.003$; $r^2=-0.603$, $p=0.001$; $r^2=-0.466$, $p=0.011$, fig 3b). The correlation with nitrogen in the soil was the weakest. Interestingly,

canopy height was strongly correlated with percentage of rock cover ($r^2=0.811$, $p<0.001$) and herbaceous cover was negatively correlated with rocks ($r^2=-0.827$, $p<0.001$, fig3b).

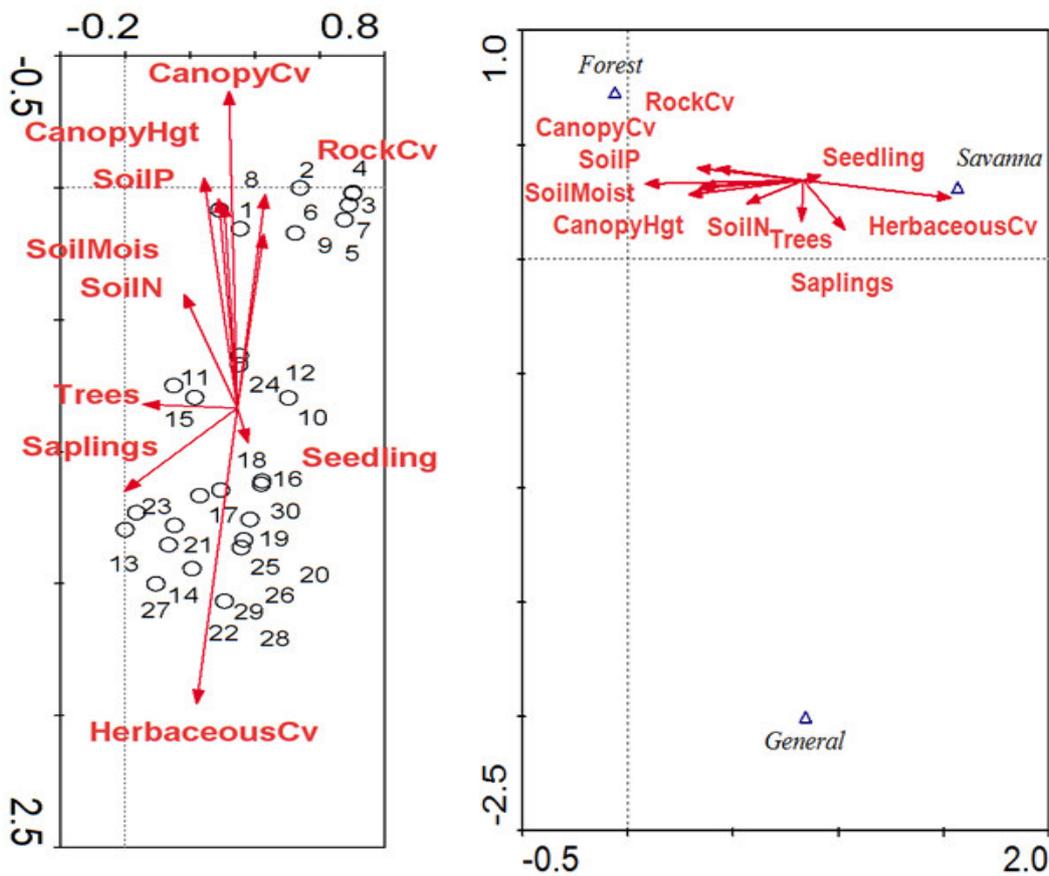


Figure 3: Ordination plot with measured biotic and abiotic factors and the plots (left) and between measured factors and the mean amount of trees per species group (right). 1-5=tall evergreen forest, 6-10=short evergreen forest, 11-15= semi-deciduous forest, 16-20 =closed savanna, 21-25= semi-closed savanna, and 26-30=open savanna.

In tall evergreen forest and low evergreen forest, savanna tree species were virtually absent (fig 4c). In semi-deciduous forest there was no difference between the amount of savanna and forest trees ($F_{1,9}=0.728$, $p=0.418$, fig 4c). In closed woodland forest trees were present, but savanna trees were dominating ($F_{1,8}=13.120$, $p=0.007$, fig 4c), and in semi-closed savanna and open savanna forest tree species were virtually absent (fig 4c). The mean amount of forest trees was strongly increasing when the canopy cover reached 50 % and the herbaceous cover was less than 30 % (fig 4a,b). The mean amount of savanna trees was highest at 15 % canopy cover and then declined when canopy cover increased (fig4a,b).

Soil analysis revealed that forest plots contained more soil phosphorus and nitrogen, moisture and organic matter than savanna plots ($p<0.001$ for all variables). Interestingly, when comparing between the three forest vegetation types no significance in the soil properties was found. Also, between the three savanna

vegetation types there were no significant differences in soil properties, although strong differences in vegetation structure existed (data not shown).

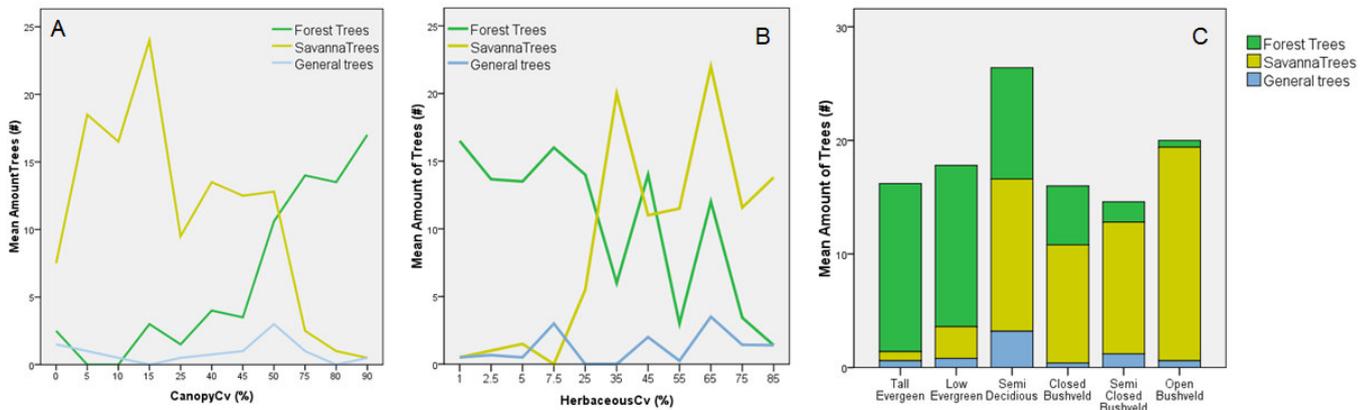


Figure 4: Mean amount of trees per type (forest, savanna or general) displayed against (a) canopy cover and (b) herbaceous cover and per vegetation type (c).

Nursery

Total Seedling Weight

Total seedling weight (TSW) of forest seedlings was less than savanna seedlings when growing with grass or when fertilized ($F_{1,52} = 4.287$, $p=0.04$; $F_{1,51}=6.786$ $p=0.012$; fig 5a). Savanna and forest seedlings reacted differently to grass ($F_{1,260} = 5.013$, $p=0.026$) and to fertilization ($F_{1,260} = 8.664$, $p=0.004$). The grass-nutrient interaction was also different for savanna and forest seedling ($F_{1,260} = 4.033$, $p=0.046$) as there was a nutrient-grass interaction in savanna seedlings, but not for forest seedlings. Savanna seedlings did not have a lower TSW than forest seedlings under low light conditions ($F_{1,73} = 1.523$, $p=0.022$, fig 5b), but higher TSW under high light conditions ($F_{1,76} = 5.820$, $p=0.018$, fig 5b). Forest seedlings also reacted differently to changing light conditions ($F_{1,149} = 4.264$, $p=0.041$).

The Combretaceae showed a strong difference when grass was added ($F_{1,57} = 4.907$, $p=0.031$). TSW of *Combretum molle* did not reduce when grass was added, however TSW of *Combretum kraussi* was halved (fig 5c). *Syzygium legatti* reacted differently to added nutrients ($F_{1,56} = 9.112$, $p=0.004$), but not to grass competition ($F_{1,56} = 3.790$, $p=0.056$) than *Syzygium cordatum* (fig 5d). The Sapotaceae showed no different reaction to grass or nutrients (fig 5e).

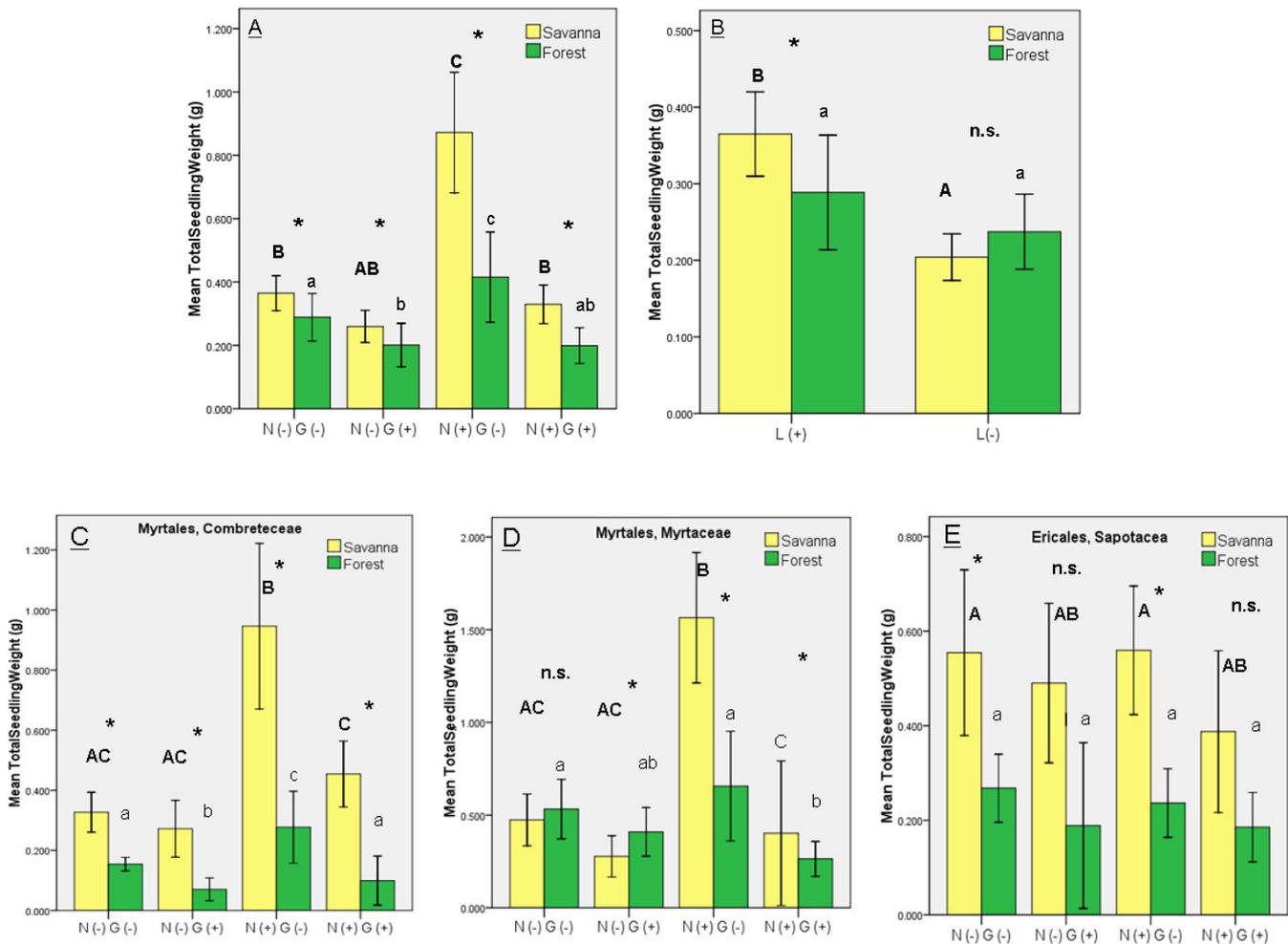


Figure 5: Total seedling weight averaged for savanna and forest species for the nutrient*grass design (a), light design (b) and per confamilial species pairs (c,d,e). Significance between species type is displayed with either a '*' or n.s. when not significant. The capital letters display significance for savanna seedlings between treatments. The small letters are used for the forest seedlings between treatments. Error bars: 95% C.I. Significance levels were tested with log transformed data.

Root mass fraction

Averaged over all treatments savanna seedlings invested more in their roots than forest species ($F_{1,336}=10.988$, $p=0.001$). This is also seen in the sun and in the shade ($F_{1,63}=4.420$, $p=0.04$; $F_{1,61}=6.809$, $p=0.11$, Fig6b), but not when nutrients or grass were added (fig 6a). Comparing within functional types and between treatments, only forest seedlings invested more in belowground biomass when grass was present and savanna seedlings did not. Both types invested more in aboveground biomass when nutrients were added ($F_{1,163}=6.110$, $p=0.014$; $F_{1,94}=9.991$, $p=0.002$). The plasticity of the functional types was not different, since there were no type*grass ($F_{1,274}=0.094$, $p=0.759$), type*nutrients ($F_{1,274}=0.726$, $p=0.395$), and type*shade ($F_{1,145}=0.014$, $p=0.905$) interactions.

Comparing confamilial species pairs in Sapotaceae, *Englerophytum megalismontanum* seedlings had a much higher RMF than *Mimusops zeyheri* seedlings, when growing in the sun, with added nutrients and in the

shade, but not when grass was present (fig 6d). There was no significant difference in the RMF in all the treatments for the Myrtaceae family (data not shown). RMF of *Croton sylvaticus* increased from 0.4 to 0.6 ($F_{1,8}=70.784, p<0.001$) when grass was added. Interestingly, the effect seen when nutrients are present disappears when both nutrients and grass are present.

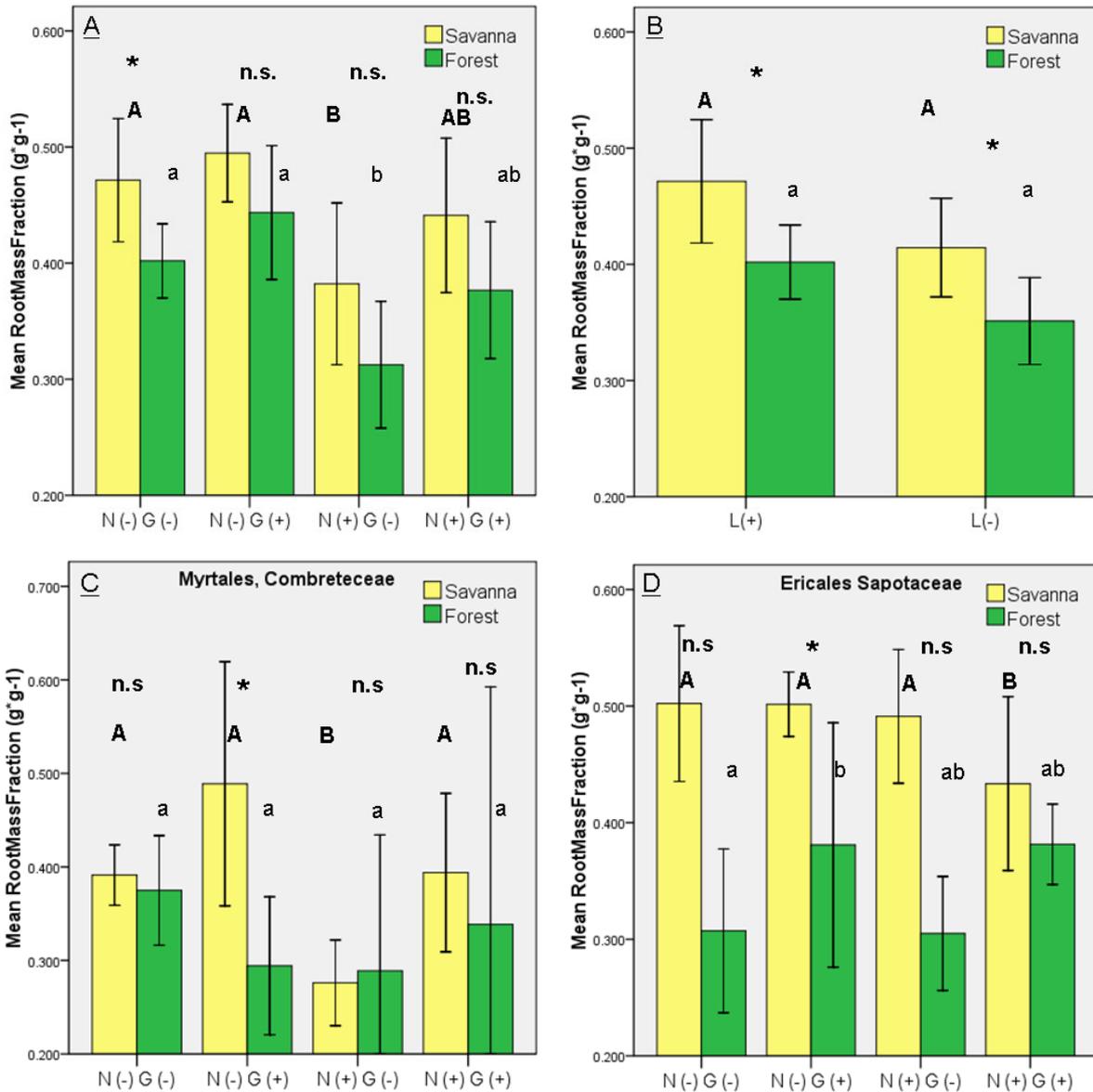


Figure 6: Root mass fraction (RMF) averaged for savanna and forest species for the nutrient*grass design (a), light design (b) and per confamilial species pairs (c,d). Significance between species type is displayed with either a '*' or n.s. when not significant. The capital letters display significance for savanna seedlings between treatments. The small letters are used for the forest seedlings between treatments. Error bars: 95% C.I. Significance levels were tested with log transformed data.

Leaf area ratio and specific leaf area

Savanna species had a higher leaf area ratio (LAR) than forest species in shaded conditions and when both nutrients and grass were present ($F_{1,61}=3.778$ $p=0.05$, fig 7b; $F_{1,45}=5.645$ $p=0.02$, fig 7a). In all other treatments there were no significant differences (fig 7a). Both forest and savanna seedlings did not change LAR in response to different light levels, and they did not respond differently ($F_{1,143}=3.511$, $p=0.063$). Specific leaf area (SLA) was not different between functional types in all the treatments (data not shown). However, they did respond differently to light levels since there was a type*shade interaction ($F_{1,137}=7.114$, $p=0.009$), so that savanna seedlings had a greater response to light and thus a greater plasticity.

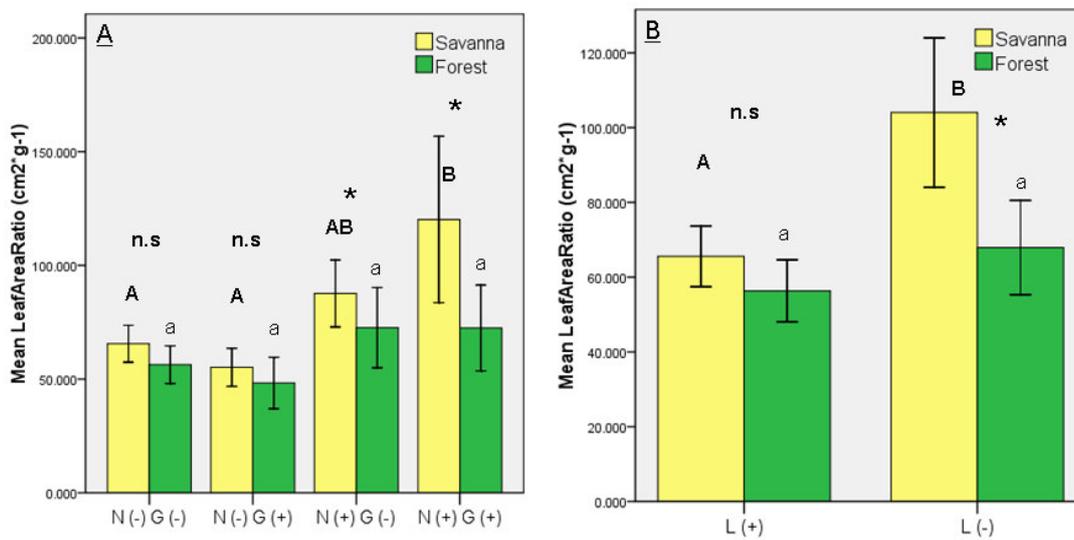


Figure 7: Leaf area ratio averaged for savanna and forest species for the nutrient*grass design (a), light design (b). Significance between species type is displayed with either a '*' or n.s. when not significant. The capital letters display significance for savanna seedlings between treatments. The small letters are used for the forest seedlings between treatments. Error bars: 95% C.I. Significance levels were tested with log transformed data.

Comparing between the confamilial species pairs only significant differences were found between the light treatments and not when nutrients or grass were present. In the sun, Myrtaceae savanna seedlings had a higher LAR than forest seedlings (fig 8b), but SLA did not differ (fig 8d). In the shade two of the three forest species had a lower LAR (fig 8a) and SLA (fig 8c).

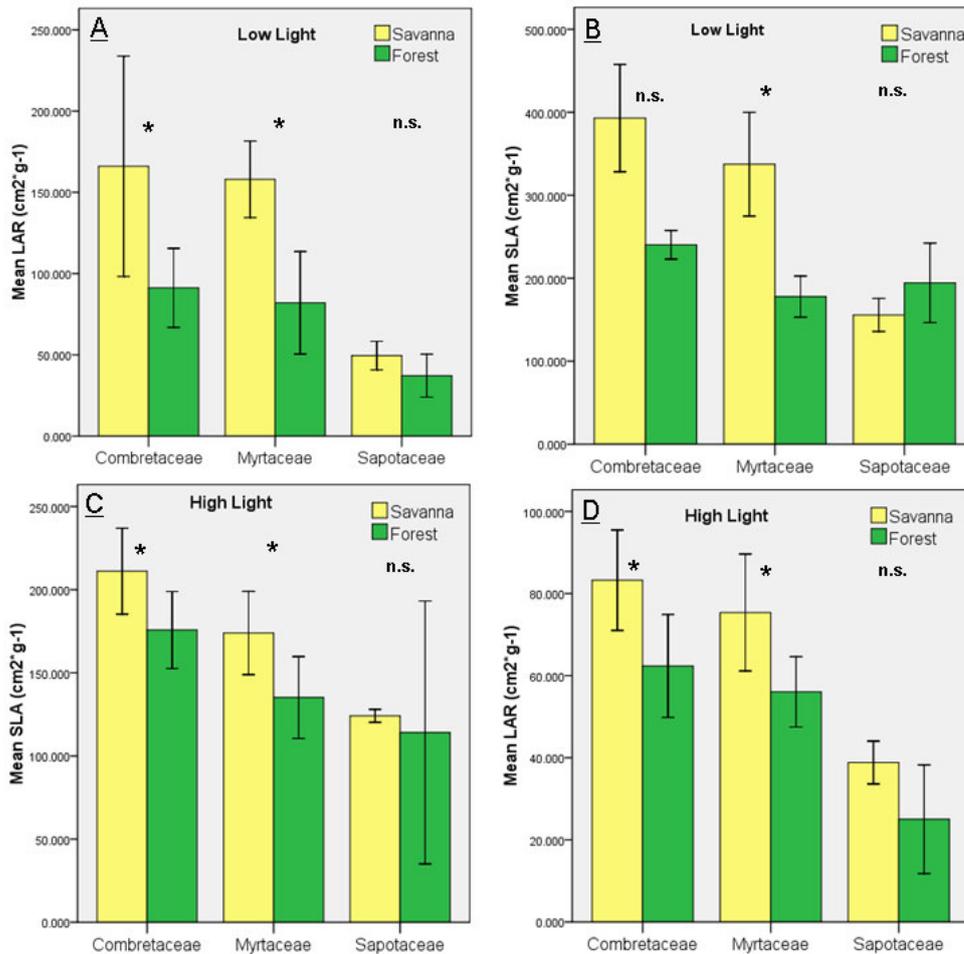


Figure 8: SLA and LAR for confamilial species pairs, height and low light treatments. Significance between functional type is displayed with either a '*' or n.s. when not significant. The letters display significance within a functional type but between the treatments. Error bars: 95% C.I. Significance levels were tested with log transformed data.

Discussion

The results show that in the savanna-forest mosaic of the Soutpansberg in South Africa, forest species and savanna species are dominant in their respective environments and few are common to both environments, similarly as in Nigeria and Brazil (Adejuwon & Adesina, 1992; Felfili & Junior, 1992). Contrary to forest-savanna boundary in Australia, tree density does not sharply decline in the transition from forest to savanna. The absence of fire for over fifteen years could explain this, since in Australia, the ecotone suffered frequent fires (Bowman, 2000). Contrary to the hypotheses, in closed savanna, savanna species are dominating and surprisingly in semi-deciduous thicket neither forest or savanna species were dominating. This is not only true for the amount of individuals but also the species diversity. It seems that forest is not advancing into savanna.

The strong correlations show that forest species advance is limited by open canopy, competition with herbaceous cover and poor nutrient availability. Interestingly, results of the nursery experiment might give further insights in above described limitation of forest advance into savannas. Savanna seedlings have a higher

total seedling weight than forest seedlings averaged over treatments. Growth of both seedling types was effected by the presence of grass, but the competitive effect of grass was higher on forest seedlings than savanna seedlings. The competitive effect of grass has already been shown for several savanna species (Riginos, 2009; Scholes & Archer, 1997), and is contrary to results that showed facilitation by grass (Anthelme & Michalet, 2009). However, this has not been shown for forest species and this is the first time that a phylogenetic independent study shows that savanna seedlings are better competitors with grasses than forest seedlings.

When fertilized, savanna seedlings had a stronger increase in biomass production than forest seedlings. This effect is however not seen in previous comparative growth analysis (Hoffmann & Franco, 2003). When both grass and nutrients were present, the effects of added nutrients were negated. Higher grass biomass, due to fertilization, resulted in a stronger competitive effect of grass on seedlings. Similarly, nitrogen addition led to increased grass biomass and therefore reduced growth of *Acacia mellifera* (Kraaij & Ward, 2006). On contrary to this result, Van de Waal et al., (2009) found that the herbaceous competition on one year old seedlings of *Colophospermum mopane* did not increase with increased nutrient availability, although herbaceous biomass strongly increased. However, in pot experiments done with recently germinated *C. Mopane*, increased grass biomass due to fertilization did stronger suppressed seedling growth (Van de Waal, 2010). This could suggest that older seedlings are less vulnerable for grass competition. However, grass has been shown to impact tree growth across all demographic stages (Riginos, 2009).

Savanna seedlings had a higher root mass fraction than forest seedlings, which might enable them to compete with grass for low water and nutrient availability, and forest seedlings for light in forests (Hoffmann & Franco, 2003). This trade-off might also explain that savanna seedlings are better competitors than forest seedlings with grass, and showed a sharper increase in biomass production when fertilized. Finally, it also allows seedlings to deal with defoliation events such as fire (Hoffmann, Orthen, & Franco, 2004) and herbivory. However, how the effect of herbivory on both savanna and forest seedlings differ remains to be tested.

Forest seedlings did not have a higher LAR and SLA than savanna seedlings, even in shaded conditions, which is contrary to our expectations, (Hoffmann & Franco, 2003) since forest trees do invest more in above ground biomass. However, when *Mimusops zeyheri*, a forest species with large cotyledons and after ten weeks only few leafs was excluded, there were no differences between savanna species and forest species over all treatments. Further, the variance in SLA and LAR was much higher than for the measured traits not involving leaf area. It could be that when growth was followed up to 150 days as in Hoffmann & Franco, (2003) results are different. A decreasing coefficient of variance is than expected in their results to confirm this statement.

Savanna seedlings showed a greater plasticity in their leaf traits, but there was no difference in plasticity in RMF, which is similar as in (Hoffmann & Franco, 2003). Savanna seedlings were expected to exhibit more plasticity since they are used to a more heterogeneous environment (Bazzaz, 1999). Above described differences in traits between forest and savanna seedlings are not always found when species are examined individually. *Acacia karroo*, a pioneer species showed high adaptability for LAR and RMF (data not shown). Both *Syzygium*

legatti and *Syzygium cordatum* did not change their RMF in all treatments. *Syzygium legatti*, the forest species of Myrtaceae, was also not effected by grass which could be explained by its larger seed size (Poorter & Rose, 2005). Further, in the shade it developed a single long stem, but in the sun it developed several shorter stems. Interestingly, this species is mostly found in low evergreen forest and transitional vegetation, so it could be an early encroaching species. Some forest species seem to be more able to invade early in savannas than other forest species, which is also reported in other studies (Hoffmann et al., 2004).

In the absence of fire, transitional vegetation of the Soutpansberg is dominated by savanna tree species. This could indicate that advance of forest into savanna, might only be possible if savanna trees provide the necessary conditions, such as shade, reduced herbaceous cover and increased nutrient availability. The strong negative correlation of forest species with herbaceous cover and the positive correlation with canopy cover reinforces this statement. This can already be seen in the seedling stage; the competitive effect of grass on forest seedlings is higher than for savanna seedlings and biomass production of forest seedlings is abated in the open sun. The ecological trade-off of forest seedlings to invest in aboveground biomass instead of belowground biomass might explain this. This can imply that closed savannas, are more sensitive to encroachment when grazed (Kraaij & Ward, 2006). Similarly, when restoring into savannas degraded forest, herbaceous cover can limit forest expansion (Gómez-Aparicio, 2009; Perrow & David, 2004). However, since no accurate data is available on how the floristic composition changed over time, conclusions are difficult to make here. Monitoring species composition, and corresponding biotic and abiotic factors over time will be interesting future research.

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