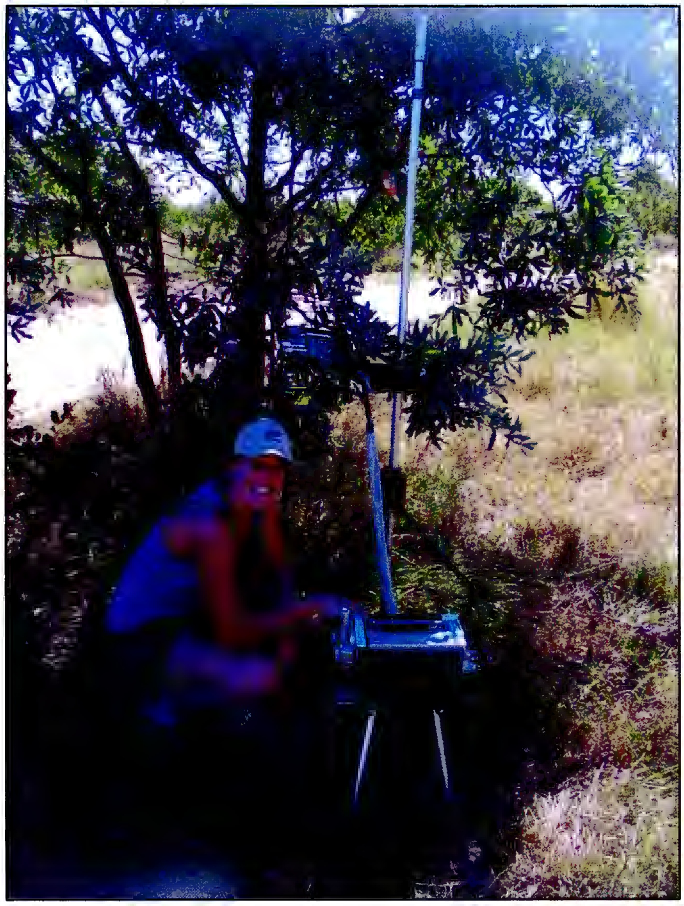
Leaf nutrient draw back as a strategy for tree-grass coexistence in the savanna biome.

By: Stephanie Williams
Supervisor: Edmund February



A dissertation submitted to the University of Cape Town, in partial fulfilment of the requirements for the award of an Honours degree in Ecophysiology.

October 2009

ABSTRACT

As there is still a major debate on how trees and grasses manage to coexist in the savanna biome, the niche separation by phenology hypothesis (Scholes and Archer 1997) was tested by observing the seasonal variation in carbon and nitrogen concentrations in leaves and young twigs of two deciduous species, *Combretum apiculatum* and *Terminalia sericea*. The study was carried out in the sub-tropical savanna biome in southern Kruger National Park, South Africa. Photosynthetic readings show a decrease in leaf activity towards the end of the growing season. There was no significant change in the carbon concentrations over the months for both species sampled but there was a significant decrease in leaf nitrogen for *C. apiculatum*. *T. sericea* experienced relatively no nitrogen draw back until a sudden drop at the end of June. Translocated nitrogen in *C. apiculatum* accumulated in large amounts in the peripheral twigs (branching order one) compared to *T. sericea*, where nitrogen only increased at the end of June in the same location. There was also a corresponding significant change in the carbon:nitrogen ratio in *C. apiculatum*, especially in the leaves, showing a decrease in palatability as nitrogen was drawn out. The results observed did not become more pronounced in site 3, which had the highest soil moisture content. Soil moisture availability therefore does not appear to influence the degree of nitrogen reabsorption from the leaves. The hypothesis proposed is that deciduous trees use stored nitrogen from their senescing leaves to leaf out earlier gaining a competitive advantage over grasses, which are waiting for nitrogen to be mineralized in the soil with the onset of the summer rain. This study therefore supports the niche separation by phenology hypothesis to explain how these two growth forms coexist in the savanna biome.

KEY WORDS: A/Ci curves, coexistence, nitrogen, savanna, translocation.

INTRODUCTION

The savanna biome is defined as a continuous grass layer coexisting with more or less densely scattered trees (Frost *et al.* 1986). While many scientists agree with this definition the reason for the coexistence has been greatly debated (Walter 1971, Belsky 1990, Skarpe 1991, Scholes and Walker 1993 & Jeltsch *et al.* 1996). The coexistence of trees and grasses is a complex interaction between many biotic (large mammal herbivory) and abiotic (rainfall and soil quality) factors mixed with the degree of varying disturbances (fire). It is still reasonably unknown as to what exactly regulates the balance between these factors so woody plants and grasses coexist without one dominating over the other, thus the "savanna problem" (Sarmiento 1984) remains unresolved. It is important to grasp the concepts of these interactions in order to understand the functioning of this biome and thus how it will be affected by global climate change, especially since this biome is anticipated to be the most ~~e~~affected by it (Sankaran *et al.* 2005). Presently three main hypotheses exist, which suggest answers to Sarmiento's (1984) coexistence problem; these include Walter's (1971) root niche separation model, Higgins *et al.*'s (2000) demographic bottleneck model and Scholes and Archer's (1997) niche separation by phenology hypothesis.

Walter (1971) suggests that grasses and trees occupy different root niches with tree roots located deeper down in the soil profile compared to the surface layer roots, which grasses occupy. Water is thus proposed as the resource determining tree-grass coexistence because there is no competition between the two for water as trees use a deeper water source and grasses use shallower water. This hypothesis has been supported (Walker and Noy-Meier 1982, Knoop and Walker 1985 & Weltzin and McPherson 1997) but also contradicted (Johns 1984, Belsky 1990 and Seghieri 1995) because a number of studies have shown that tree seedlings still have their roots in the top horizon competing with grasses in the establishment stage (Medina and Silva 1990, Mordelet *et al.* 1997).

Higgins *et al.* (2000) proposed an alternative model, which used a combination of climatic variables and disturbances, such as fire to explain tree demography. They suggest that tree seedling recruitment is limited by rainfall as grass biomass increases as rainfall increases reducing the amount of space available for seedling establishment. Fire, on the other hand prevents tree recruitment into adult size classes although with an increase in fire, a decrease in grass biomass results and the probability of tree seedlings establishment in the open space is increased (Higgins *et al.* 2000). Rainfall and fire are primarily the controlling factors in this model and nutrient limitation or accessibility is not mentioned consequently although this model does incorporate a variety of variables, the interpretation of models in the context of different scenarios needs revision (Sankaran *et al.* 2004). There is also the ongoing debate as to whether models are in fact successfully capturing the complexity of savanna systems therefore casting doubt on this hypothesis (Belsky 1990; House *et al.* 2003).

The third hypothesis for savanna tree-grass coexistence is the niche separation by phenology hypothesis, which was investigated by Scholes and Archer (1997). Phenology is the study of plant life cycle events and the influence of seasonal variations on these cycles. Many savanna trees are deciduous meaning they lose their leaves in winter. In winter there is little rainfall therefore it is energetically costly for trees to maintain their leaves throughout the season. Nutrient reabsorption or the withdrawal of nutrients from senescing leaves and their storage in woody parts is a common phenomenon in deciduous trees (Kramer and Kozlowski 1979, Chapin and Kedrowski 1983, Gray 1983 & Santa Regina *et al.* 1997). Birk & Vitousek (1986) showed evidence of nitrogen retranslocation in senescing leaves of pine forests in South Carolina where whole canopy retranslocated nitrogen ranged from 35kg.ha^{-1}.yr^{-1} to 95kg.ha^{-1}.yr^{-1}, which is a huge amount of nutrients saved by the tree. The niche separation by phenology hypothesis investigates whether deciduous trees withdraw their nutrients from their leaves into their branches at the end of the growing season. In this way trees obtain a competitive advantage over grasses as they are able to produce new leaves before nutrients become available to the grasses at the beginning of the wet season (Scholes and Archer 1997 & Williams *et al.* 1997). The trees thus have exclusive access to resources compared to grasses, which then have to be superior competitors when they leaf out later (Ryan and Bormann 1982 & Kemp 1983). It is proposed that the trees are utilizing one source of nitrogen (pulled back from their leaves during the previous season) while the grasses are utilizing their nitrogen later in the season from newly mineralized nitrogen in the soil. Davis *et al.* (1998) supports the niche separation by phenology hypothesis by showing that in resource-limited environments, establishment and recruitment of seedlings are more limited by availability of resource rather than competition.

I investigated this hypothesis using two species of winter deciduous trees in the Kruger National Park (*Combretum apiculatum* Sond. and *Terminalia sericeaa* Burch. Ex DC). Firstly, I test whether nutrients (nitrogen and carbon) are reabsorbed from the leaves into the branches at the end of the growing season. I also determined where in the tree these nutrients are stored. I focus on nitrogen because it is generally one of the most limiting nutrients in savanna ecosystems (Sollins *et al.* 1980, Chapin *et al.* 1987, Norby *et al.* 2000 & Craine *et al.* 2008). Nitrogen is also an element that is most likely to be reabsorbed by the tree as reabsorption of other elements has only been found in insignificant amounts in previous studies (Ryan & Bormann 1982). As plant available moisture has been suggested as one of the most important factors governing the structure and function of savannas (Walker & Landridge 1997), three sites along a slight rainfall gradient were compared to see if the efficiency of nitrogen retranslocation was correlated with rainfall.

METHOD & MATERIALS

Study Site and study species

The trees used for this study were located in the southern part of the Kruger National Park (KNP), Mpumalanga Province, South Africa (Fig. 1). The area occurs in the lowveld bushveld zone where the nutrient poor soils are derived from the underlying granite (Du Toit *et al.* 2003). The uplands consist of open tree savanna and are dominated by *Terminalia sericea* and *Dichrostachys cinerea* with relatively few low shrubs. A dense grassy layer exists, which is dominated by *Hyperthelia dissolute* and *Elionurus muticus*. The narrow bottomlands in the Pretoriuskop region are dominated by *Acacia* species (Mucina and Rutherford 2006). The climate is sub-tropical with the rainy season occurring between November and March. Temperatures range between a maximum of 32.6 °C in January to a minimum of 5.6 °C in June (Du Toit *et al.* 2003). Trees from three sites located between Skukuza and Pretoriuskop rest camps (25.07°S 31.55°E; 25.09°S 31.38°E; 25.09°S 31.38°E), were sampled. The sites were also situated along a rainfall gradient with the site closest to Skukuza receiving an average monthly rainfall of 0.04mm, the middle site 0.06mm and the site closest to Pretoriuskop 0.08mm. The two species chosen for this study include *Combretum apiculatum* (Sond.), commonly known as the red bushwillow and *Terminalia sericea* (Burch. Ex DC), commonly known as the silver cluster-leaf. Both species are drought deciduous, losing their leaves in winter.

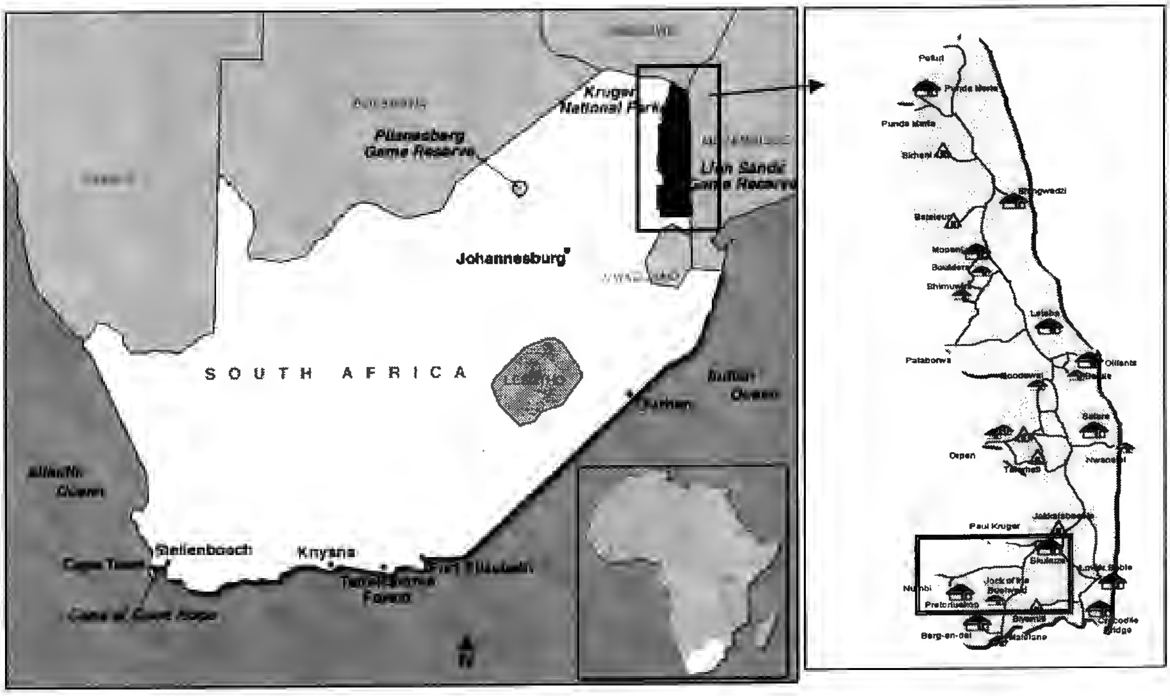


Fig 1: Map of The Kruger National Park showing the location of the study sites.

Nutrient Drawback

At the three study sites three *Terminalia sericea* and three *Combretum apiculatum* trees were tagged. Leaves from the first four branching orders were collected at the end of each month from March to June (Fig. 2). Approximately 4cm of wood was collected from each of the first three branching orders using a pair of secateurs. As the wood from branching order four was too thick to cut, this wood was collected using a 12V cordless drill (AEG, Germany) with a 16 mm diameter wood drill bit. Soil moisture was obtained using an EC-5 soil moisture probe (Echo) attached to a Campbell Scientific CR10x data logger

(Decagon Devices, Pullman, Washington, America). Values were averaged over one hour. All samples were dried to a constant weight at 70°C in a forced convection oven (Scientific, Series 2000 oven, South Africa) and the bark and pith removed. Leaves and wood were ground to a fine powder using a Retsch MM200 ball bearing mill (Retsch, Haan, Germany). Leaf and wood percentage C, percentage N and C:N ratios were de*Terminalia* ed using a Thermo Finnigan Delta plus XP Mass Spectrometer coupled with a conflo III device to a Thermo Finnigan Flash EA1112 Elemental Analyser with automatic sampler (Thermo Electron Corporation, Milan Italy).

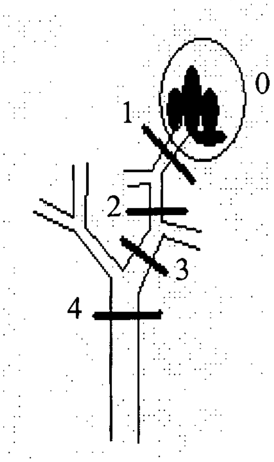


Fig. 2: Diagram showing location of samples obtained. 0- leaves and 1-4 are branching orders with order 1 closest to the leaf and order 4 the furthest from the leaf.

Gas exchange

In the field an infra-red gas analyser (LICOR 6400 Inc., Lincoln, USA.) was used to determine photosynthetic rates (A) at increasing carbon dioxide concentrations [C_i] (50-580 ppm) for one *Terminalia sericea* and one *Combretum apiculatum* at each of the three sites (n = 6). Leaf temperatures were set at 25°C and leaves illuminated using the built in red-blue light source at 1500μmm^{-2}s^{-1}. Leaf vapour pressure deficits were maintained at 1.5kPa ±0.1. Fully expanded leaves, exposed to direct sunlight were used. The equations used in this analysis to calculate the A/Ci curves were taken from Long and Bernacchi (2003). The curves were plotted in Microsoft Excel. One of the main assumptions for interpreting these A/Ci curves is that all leaves behave in the same manner at the exact same time (Sharkey 2007). There are three main processes, which affect the rate of photosynthesis (A). These include the rate of RuBP carboxylation, the rate of photorespiration and the light use efficiency. The A/Ci equation assumes all these processes are equal in all leaves.

Statistical analysis

One-way ANOVA's were performed on the original data in STATISTICA 8 to test for a decrease in leaf photosynthetic rates from March through June and also differences in soil moisture content between sites. A regression analysis was performed in Microsoft Excel (2003) to test for significant correlations in the drop in leaf nitrogen and change in carbon: nitrogen ratios from March through June in both species. A factorial ANOVA and a post-hoc Tukey HSD test were performed to determine differences in nitrogen and carbon drawback from the leaves, the change in C:N ratio and to test for significant differences in the location of the nitrogen in and how it changes over time.

RESULTS

Nutrient drawback

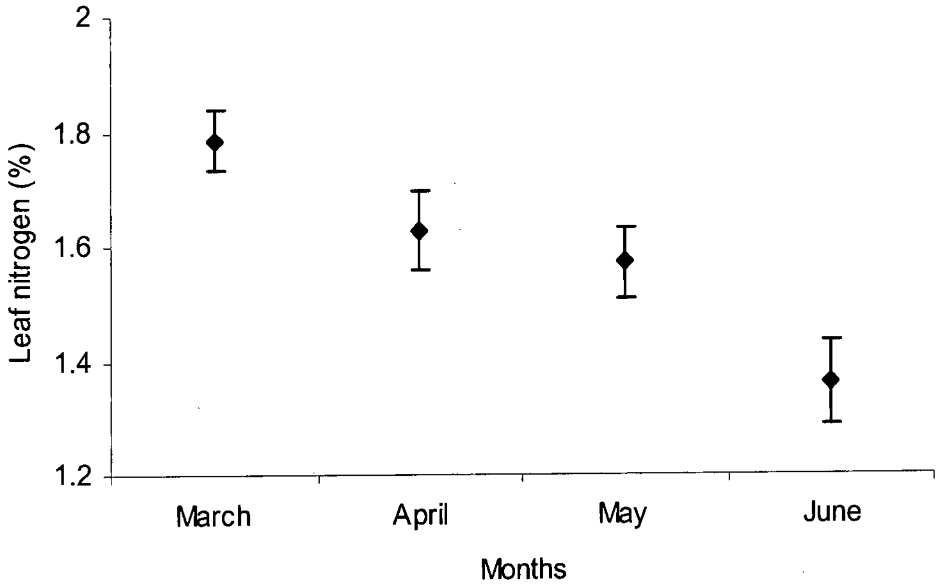


Fig 3: Means and standard error for leaf nitrogen for the last four months of the growing season for nine *Combretum apiculatum* trees ($r^2 = .96$, $P < .05$).

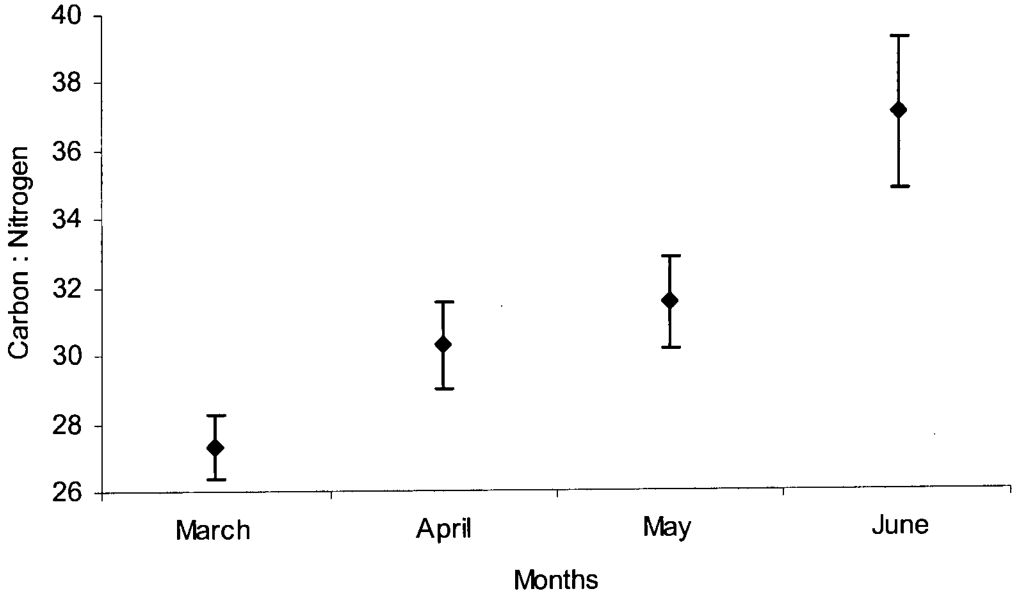


Fig 4: Leaf C:N ratios and standard error for nine *Combretum apiculatum* trees for the last four months of the growing season ($r^2 = .98, P < .001$).

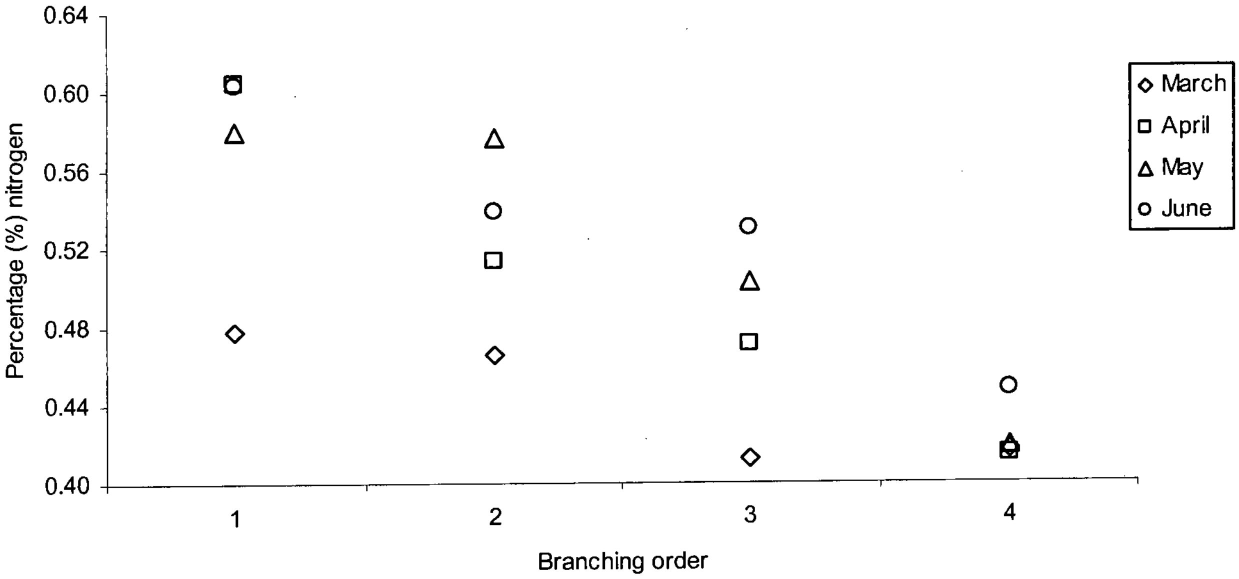


Fig 5: Total nitrogen for the four branching orders of *Combretum apiculatum* for the last four months of the growing season, showing the increase in nitrogen below the bud (order 1) at the end of the growing season.

All nine individuals from the three sites were combined to receive an average nitrogen and carbon abundance as well as to determine changes in carbon:nitrogen (C : N) ratio over time. There is a decrease in leaf nitrogen content in *Combretum apiculatum* from March (1.8%) through to June (1.4%; Fig. 3). Tukey's HSD test showed a large drop in percentage leaf nitrogen from March to June (p<0.001) and even from April to June (p<0.05). There was no significant change in *Combretum apiculatum* leaf carbon content over time (p>0.05), which is backed up by the increasing C:N ratio (Fig. 4) as nitrogen decreases in the leaves. There is a particularly large ratio increase at the end of June (p<0.001) correlating to the significant drop in leaf nitrogen. There is a decrease in nitrogen from branching order one through to branching order 4 in *Combretum apiculatum*, where there is a large increase in nitrogen in the 1st branching order after March (p<0.001). There was significantly less nitrogen in the trunk of the tree (branching order 4; p<0.001) and the 1st and second branching orders had considerable more nitrogen than the other locations (p<0.001; Fig. 5).

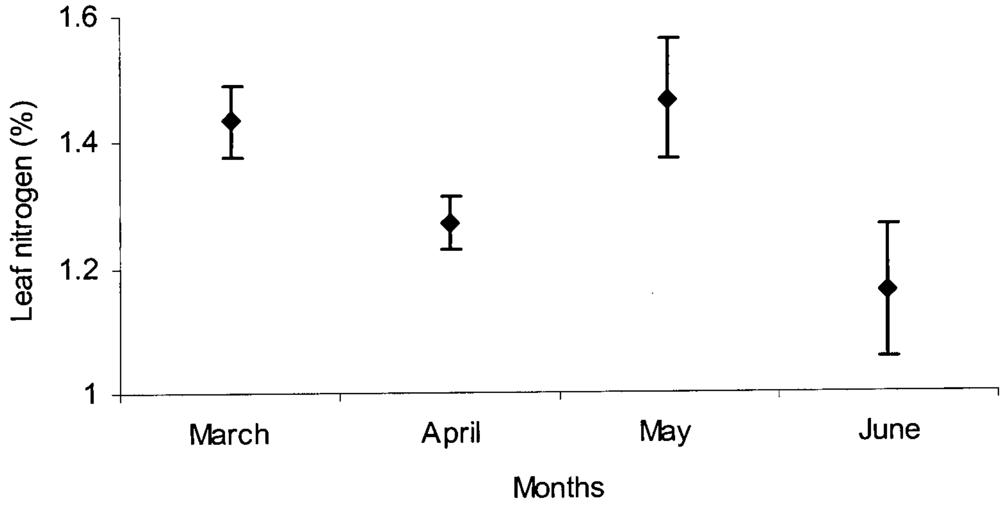


Fig 6: Average change in leaf nitrogen content with standard error bars for nine *Terminalia sericea* trees for the last four months of the growing season (r^2 = .31, P < .05).

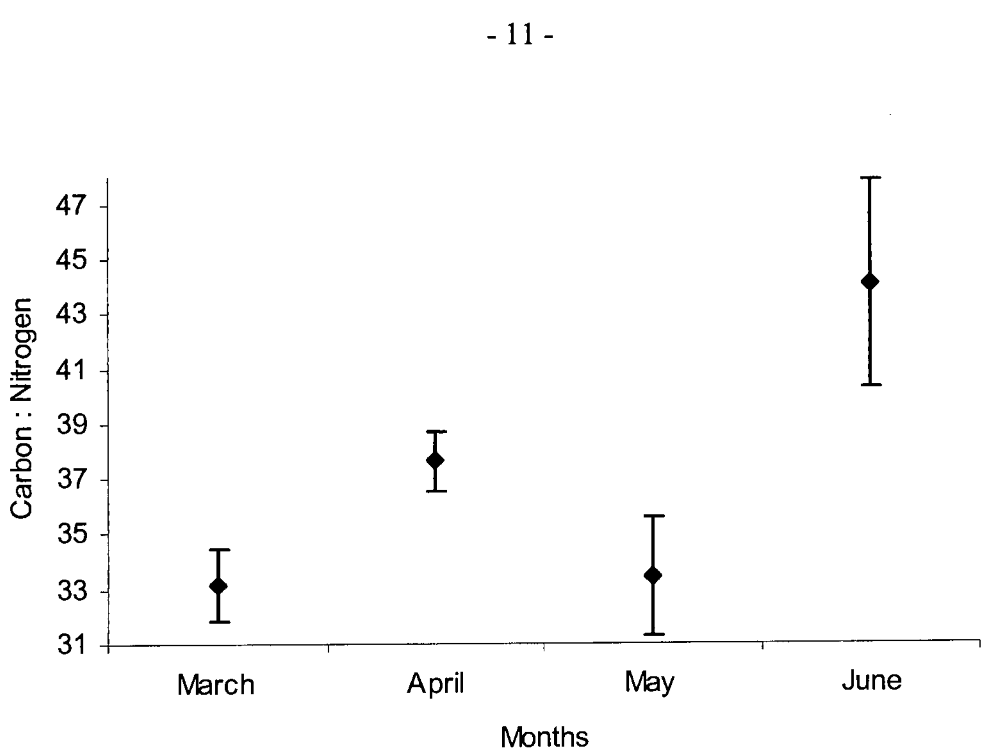


Fig 7: Average change in leaf C:N ratios with standard error bars for nine *Terminalia sericea* trees for the last four months of the growing season ($r^2 = .52, P < .05$).

Fig. 8: Total nitrogen for the four branching orders of *Terminalia sericea* for the last four months of the growing season, showing the increase in nitrogen below the bud (order one) at the end of the growing season.

There were no significant differences in total leaf nitrogen content from March through to May for *Terminalia sericea* (Fig.6). There was, however, a sudden decrease in leaf nitrogen at the end of June, which was significantly different to the percentage in March ($p<0.05$). Similar to *Combretum apiculatum* there were no significant difference in the carbon content through time. C:N ratios for *T. sericea* were also not significantly different from March through to May until the sudden increase at the end of June ($p<0.01$; Fig 7). The trend for a decrease in nitrogen from the 1st branching order through to the 4th order is not as clear for *T. sericea* (Fig. 8) as it is in *Combretum apiculatum* (Fig. 5). By the end of June, however, nitrogen concentrations were significantly higher in the terminal branch (order one) compared to March ($p<0.001$).

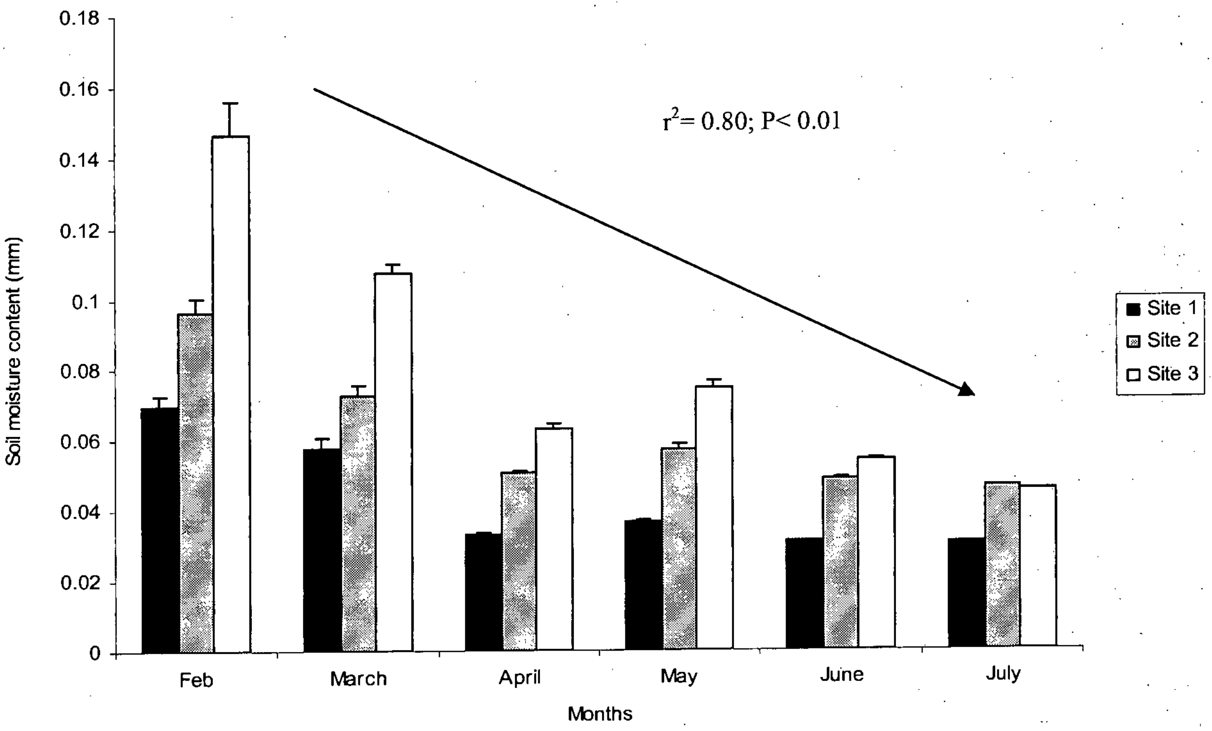


Fig. 9: Average monthly soil moisture (mm) content (+SE) at the three sampled sites showing the significant decrease through time.

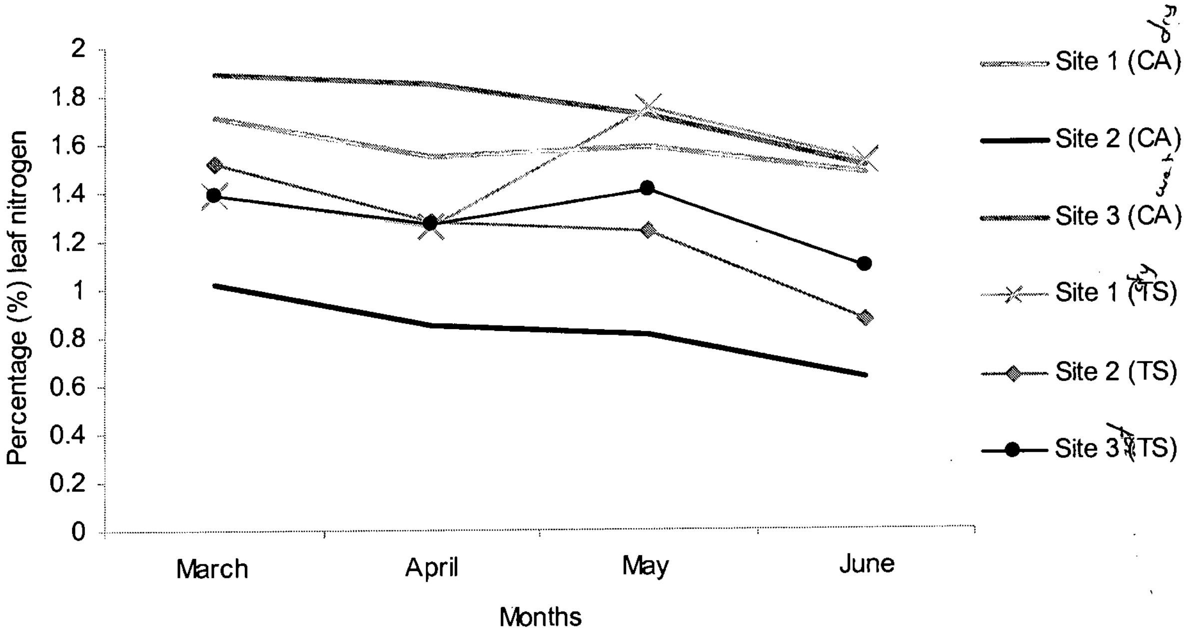


Fig 10: Percentage leaf nitrogen of *Combretum apiculatum* (CA) and *Terminalia sericea* (TS) for the three sampled sites for the last four months of the growing season.

At the study sites soil moisture decreased from February through to July (Fig. 9). Site 1, closest to Skukuza, had the driest soils; site 2 intermediate and site 3 had the most available water. There was no nutrient drawback difference between the three sites according to the different soil moisture contents as *Combretum apiculatum* species in site 1 and site 3 had little difference in their leaf nitrogen drawback amounts (Fig. 10) even though these two sites have the greatest contrasting soil moisture results. Site 2 has intermediate soil moisture and the lowest amount of nitrogen in the leaf at the beginning and end of the growing season. Similar results were observed for *Terminalia sericea* with the trees in site 2 having the least nitrogen drawback showing that nutrient withdrawal from the leaves is not dependent on soil moisture content. Photosynthetic rates reflect the decrease in soil moisture over the growing season (Fig. 11 & 12). *Combretum apiculatum* has the highest photosynthetic rate in March reflecting the high soil moisture. As soil moisture decreases so does the photosynthesis (Fig. 11). *Terminalia sericea* does not exhibit the same trend. There is however, significantly lower photosynthetic activity in June compared to the other months where the soil moisture is the lowest ($p<0.005$).

Gas exchange

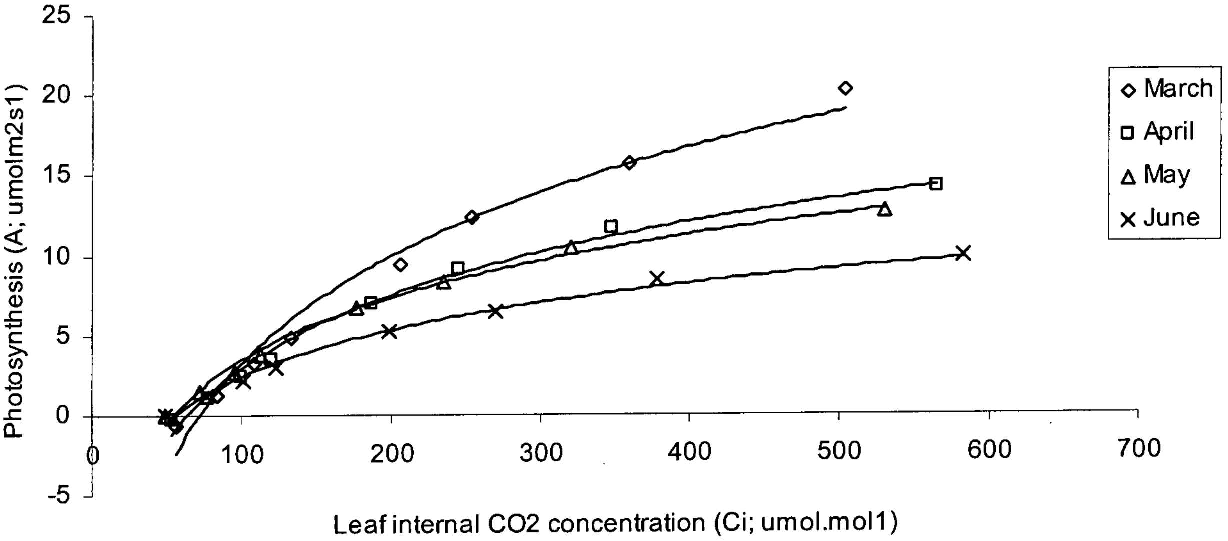


Fig 11: A/Ci curve of *Combretum apiculatum* for the months March to June. Each point represents an average for nine plants.

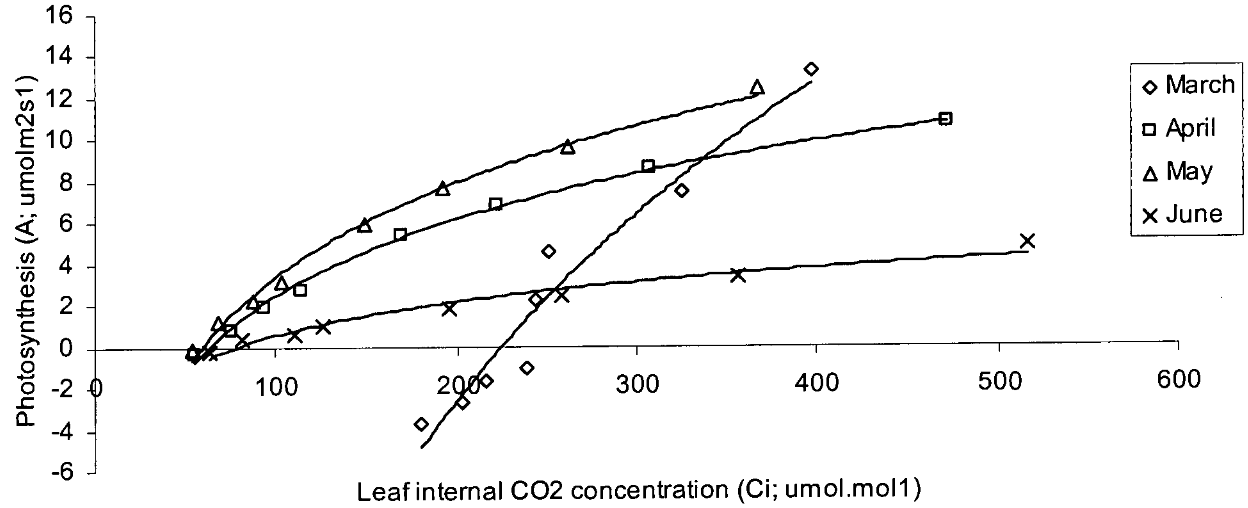


Fig 12: A/Ci curve of *Terminalia sericea* for the months March to June. Each point represents an average for nine plants.

DISCUSSION

In savannas, tree-grass coexistence has been extensively researched and reviewed (Walter 1971, Belsky 1990, Scholes and Archer 1997, Higgins *et al.* 2000 & Sankaran *et al.* 2004). The 'savanna problem' proposed by Sarmiento (1984) reiterates the fact that trees have managed to persist in an environment where grasses are highly competitive. The ability for grasses to swiftly utilize resources in the surface soil horizon and grow rapidly gives them a major competitive advantage over the slower growing trees (Bond 2008). With fire as a frequent disturbance, grasses are burnt away allowing space for the tree seedlings to establish (Higgins *et al.* 2000 & Bond 2008). It is once trees have escaped this frequently burnt level that the niche separation by phenology hypothesis, as proposed by Scholes and Archer (1997) can be used to explain how trees are coexisting with grasses. Trees are able to leaf out earlier compared to grasses, by utilizing a reserve nutrient source, which is drawn back from the senescing leaves at the end of the growing season. Although this study focused on deciduous trees, evergreen species limit their leafing in the dry season compared to the high foliage cover produced in the rainy seasons (Seghieri *et al.* 1995) therefore the niche separation by phenology hypothesis applies to both types of life history strategy.

The ability of deciduous trees to withdraw nutrients (carbon and nitrogen) from their leaves and store it in an accessible location until the next growing season, was the main focus of this study. This study shows that there is a drop in leaf nitrogen (N) and an increase in N in branching order one in *Combretum apiculatum* confirming the N retranslocation hypothesis especially for this species. Leaf N withdrawal from *Terminalia sericea* was not as clear as *C. apiculatum*, but a sudden drop occurs at the end of June. Bernhard-Reversat (1982) working with *Balanites aegyptiaca* in the savannas of Northern Senegal, showed that a drop in leaf nutrients was only found just before leaf abscission. This could be applied to *T. sericea* suggesting that it is losing its leaves later in the season compared to *C. apiculatum*. The N in the leaves is a vital element for the photosynthetic processes as some glucose manufactured by photosynthesis needs to be turned into protein. Approximately 75% of N in the plants leaf is invested in photosynthesis (Chapin *et al.* 1987) therefore a decrease in leaf N should be reflected in a decrease in photosynthetic capacity. This decrease was observed in the gas exchange measurements for both species. This correlation is especially evident in *T. sericea* as the sudden decrease in leaf N correlated well with the drop in photosynthetic rate at the end of June. The concentration of nitrogen in the leaves differs between species in order for trees to coexist together (Simioni *et al.* 2003) and so would the duration of leaf retention in different species. There was however, no leaf carbon (C) withdrawal from either species, illustrating that C is not limiting in this area and does not have to be stored for utilization the following growing season. Kobe (1997) suggested that deciduous trees store carbohydrate to gain a head start over others the following growing season but, the deciduous species in this study are not drawing carbon out of their leaves as much as they draw back nitrogen. Since C concentration did not differ throughout the sample time one can use the same assumption as Norby *et al.* (2000), that there was no shift from leaf carbohydrates to lignin.

As there is evidence of N draw back, one should question where exactly that N is being translocated to. In a study using a number of *Acacia* species, Bernhard-Reversat (1982) did not find a gradient of N in the twigs as the trees dropped their leaves, although a decrease in the amount of leaf N was observed. The explanation given was that the N was being used for wood growth but in this study, one can correlate the drop in *C. apiculatum's* leaf N with the accumulation of N in different locations down the branching orders throughout the growing season. Similarly Santa Regina *et al.* (1997) found a slight nitrogen concentration increase in the branch of *Quercus pyrenaica* just before leaf senescence. By tracking the location of N from the leaf tips (order one) through to the main trunk (order four), it was very interesting to observe the apparent accumulation of N in branching order one, as this is a clear statement of energy conservation. During the springtime flush of growth, intense cellular growth creates a high demand for nutrients, particularly nitrogen. The stored pool of nitrogen therefore needs to be readily available permitting a higher rate of growth and not held back in the main trunk where it would be energetically wasteful to acquire (Bernhard-Reversat 1982, Ryan and Bormann 1982). Nutrients immediately available with the first rains in the surface soils can be quickly acquired by the trees whose leaves are already young and requiring a nutrient source.

Tree fine roots allow immediate access to this source, which is later utilized solely and more competitively, by grasses.

The implication for this storage ability and accessible locality of reabsorb N provides better insight into the tree-grass coexistence theory. Once the initial division of resources by the two growth forms has been established the interaction of other factors suggested by Higgins *et al.* (2000), such as climate, fire and herbivory, are then the major contributing factors controlling the woody: grass ratio in the long term. It is thus proposed that access to reserved nutrients in deciduous trees, specifically nitrogen, is one of the "drivers" described by Jeltsch *et al.* (1996) in savanna tree-grass co-existence. My results agree with Knoop and Walker (1985) who show that the tree: grass ratio is mainly controlled by environmental conditions in addition to fire. Keretetse (2009 unpublished) show that at my study site trees leaf out in October before the rains or grasses, which start leaving out in November. Keretetse (2009 unpublished) also shows that nitrogen mineralization occurs in a pulse at the beginning of the wet season, which the trees can utilize immediately due to the presence of their new leaves. Scholes and Archer (1997) also noted the C4 grasses peak flush only occurring a few months after the onset of the rainy season. If the species sampled in this study already have an available N source, the onset of summer rain would trigger a high growth rate early in the season, allowing them to reach a much better competitive advantage over grasses and therefore tree-grass coexistence is possible because of the niche separation by phenology hypothesis. I propose storage of nutrients, essentially nitrogen in this case and carbon in the case of saplings or Gullivers (Bond and van Wilgen 1996), allow trees to co-exist in an ecosystem where grasses would otherwise dominate thus substantiating the niche separation by phenology hypothesis. Trees have exclusive access to this high nitrogen source much earlier in the season in preparation for rapid leaf expansion rates.

Comparison of sites along the rainfall gradient showed no significant trend for increased N drawback with less soil moisture as expected. As nutrients are only accessible when dissolved in water, the tree would have to reabsorb more nutrients from the drier soils, but this was not the case. This suggests that these species are becoming less dependent on environmental characteristics but the element of scale needs to be incorporated. Although all sites were on equal soil substratum, specific trees could be influenced by herbivore distribution ranges, termites and also location along the catena (Coughenour *et al.* 1990). If the site was located at the bottom of a catena, there would be more nutrients available to the trees, even if that site had less soil moisture. The impact of rainfall on the reabsorption of nutrients in deciduous savanna trees therefore needs to be looked into more accurately taking into account scale as a major influence. Perhaps a larger rainfall gradient range should be chosen as the sites in this study were relatively close together.

CONCLUSION

Savannas are found throughout the world in a variety of climates and topographies thus it is likely that it is not only one factor contributing to their existence. However, there has always been a need for nitrogen and the accumulation of this element in branching order one of deciduous trees in savannas suggests the importance of it in the "savanna problem" (Sarmiento 1984). The accuracy of many spatial models may also be improved so long term data on the changing ecosystem can be predicted. Manipulation of resources, namely nitrogen, by these two deciduous trees gives them a head start over the grasses at the onset of the rainy season. A faster establishment and growth rate is therefore achieved during their early flush of new leaves, before the grasses reach their peak competitiveness. Deciduous trees should be equally competitive as grasses when the summer rains arrive and thus coexist through the niche separation by phenology hypothesis first proposed by Scholes and Archer (1997) and supported in this study.

ACKNOWLEDGEMENTS

I would like to thank Edmund February for the project idea, advice and supervision of project. My fieldwork would not have been possible without Henri Combrink, based at Skukuza Scientific Services, who also collected rainfall readings for me. I would also like to thank Laurence Kruger for organizing accommodation on such short notice for my fieldwork, Iain Newton in the isotope lab and thank you to the National Research Foundation for funding this project.

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