

# Stable isotope analysis as an indicator of tree and grass rooting depth in a mesic African savanna



Sam Jack

Supervisor: Dr. Edmund February

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KD JACK

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## Abstract

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This enclosure-based study in a mesic southern African savanna investigated the validity of the Walter Hypothesis, which states that trees and grasses root at different depths and subsequently exploit different soil moisture layers. Root samples were extracted from vertical soil profiles in open and under canopy cover and analysed by means of carbon nitrogen isotopes. While  $\delta^{15}\text{N}$  showed little pattern due to complexities in the fractionation process,  $\delta^{13}\text{C}$  revealed that tree and grass rooting depths did not adhere to separate niches as proposed by Walter (1971). Root material from both growth forms occurred throughout the profile but concentrated within the upper 20-30 cm. Significantly higher densities of tree fine roots were found under canopy cover than in the open. Grass rooting followed similar patterns in both open and canopy sites, only dominating in the upper 30 cm of the open sites. Contrary to what was expected, tree fine root density increased in the top 20 cm of the open treatment, possibly due to the lack of constraining factors such as herbivory and fire within the enclosure. It is postulated that woody cover may become increasingly dominant over time due to the relatively high rainfall in the region.

## Introduction

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Savannas are characterized by the unusual coexistence of trees and grasses. This seemingly antagonistic relationship between different growth forms has been the basis of extensive study, going as far back as the 1920's (e.g. Howard, 1925). As a result there are numerous definitions for savanna. The most accepted seems to be that of Frost *et al* (1985), who described savannas as tropical or near tropical summer rainfall ecosystems that are characterized by a continuous herbaceous matrix in which trees form a discontinuous covering.

Savannas ecosystems are globally important for several reasons. Not only do they cover a fifth of the world's land surface, but they are also home to the majority of earth's human population (Frost *et al*, 1985; Scholes & Archer, 1997; Sankaran *et al*, 2005). Consequently much of their area is utilized as rangelands for livestock (Sankaran *et al*, 2004; Sankaran *et al*, 2005). Despite their ecological and economic importance, the mechanisms which lead to the co-dominance of trees and grasses remain poorly understood.

The labour intensive process of digging up root material has meant that the above-ground component historically received far more attention (Mordelet *et al*, 1997). Some studies found that tree canopies had a positive effect on understorey production due to associated reductions in water stress, decreased temperatures and increased nutrient concentrations (e.g. Belsky, 1994; Weltzin & Coughenour, 1995). Others have provided evidence to the contrary (or: support for the converse). For example, in the Lamto savanna, Mordelet (1993) found that carbon assimilation rates were 5 to 6 times lower for leaves shaded by the canopy and Mordelet & Menaut (1995) found lower grass biomass under tree canopies. What seems to be important is the degree and duration of shading (Mordelet & Menaut, 1995), the density of the canopy being inversely related to grass production.

The effects of grasses on trees are similarly variable, but what is clear is that the regulation of above-ground woody biomass can occur both directly, as competition for light, or indirectly as increased fine fuel load, which limits tree recruitment and establishment through fire (Scholes & Archer, 1997).

However, while above-ground structures are important, soil water and soil nutrient availability are thought to be the key determinants of the tree-grass assemblage (Walter, 1971; Frost *et al*, 1985). These two factors control the expression of the above-ground elements, and therefore influence the effects of herbivory and fire. Specifically, rainfall has been shown to be well correlated with woody cover; an increase in the former resulting in an increase in the latter, and vice versa.

This is supported by the most recent work by Sankaran *et al* (2005) who demonstrated, with the aid of 854 sites spread throughout Africa, that maximum woody cover was constrained by mean annual precipitation of  $650 \pm 134$  mm. This suggests a shift away from demographic bottleneck models (e.g. Higgins *et al*, 2000; Gardner, 2006), which have provided considerable insights in the tree-grass problem, especially with respect to variable impacts by disturbance factors on the different life history stages of trees (Higgins *et al*, 2000).

Numerous underlying assumptions and lack of applicability across a wide range of savanna environments is a short-coming that precludes the adoption of the bottleneck model over more traditional competition based models based on temporal or spatial niche separation theory (Sankaran *et al*, 2004).

Walter (1971) was the first to put forward a hypothesis explaining tree-grass co-existence. He proposed that trees and grasses were exploiting different layers of the soil horizon and thus competitive exclusion was negated by niche differentiation. According to his theory shallow rooted grasses occupied only the uppermost soil layers where they out-competed trees due to increased root density and more effective water absorption. In fact, he believed grasses were the controlling growth form, and trees were “merely

tolerated" (Walter, 1971). Trees, on the other hand, had exclusive access to the lower soil layers (Walter, 1971).

Walker & Noy-Meir (1982) formalized Walter's (1971) competition equilibrium hypothesis and this model has been partly applied to a wide range of savanna habitats (e.g. Knoop & Walker, 1985; Sala *et al*, 1989) with varying degrees of success (Mordelet *et al*, 1997). These and other (e.g. Belsky, 1994) empirical studies have subsequently proved that this phenomenon cannot be explained by simple niche differentiation and that tree and grass roots overlap greatly.

However, a major short-coming of these studies has been the techniques employed to differentiate between roots of the two growth forms. In most cases tree and grass root density was determined by visual counts within a standard area at different profile depths. A brief explanation of the procedure used to identify and quantify tree and grass roots will reveal two areas of concern. The depth profile is prepared by careful smoothing of the vertical surface, after which a certain amount of soil material is brushed away (Knoop & Walker, 1985) or washed away with a pressurized hose (Belsky, 1994). This could lead to losses of fine root material (Mordelet *et al*, 1997) which are the most significant roots for both trees and grasses in acquisition of water and nutrients.

Exposed roots were then 'identified' according to their morphologies as either grass or tree roots (Mordelet *et al*, 1997). This is problematic, since fine roots (>2 mm) are not easily identifiable and may have resulted in misidentification. Since it would be impractical to follow the root from its source, more accurate techniques have been developed utilizing advances in stable isotope technology. < ?

The use of stable carbon isotopes  $^{13}\text{C}$  and  $^{12}\text{C}$  in ecological applications has burgeoned since the first studies of photosynthetic fractionation in the early 1970's (Smith & Epstein, 1971). Since then  $^{15}\text{N}$  and  $^{14}\text{N}$  have been added to the suite of isotopes that can be used to investigate tracer and process information (Peterson & Fry, 1987). Stable carbon isotope  $^{13}\text{C}$  is especially popular due to the relative ease of interpreting the

fractionation process. Conversely, more factors influence the relative expression of the  $^{15}\text{N}$  isotope. It is consequently more complex and correct interpretation usually entails controlled laboratory conditions in order to quantify measurements of biological  $\text{N}_2$  fixation (Handley & Raven, 1992; Lajtha & Michener, 1994).

Carbon isotopes are especially useful in the context of savanna vegetation because species from the two dominant growth forms also happen to have different photosynthetic pathways: trees use the Calvin cycle and are thus C3, while grasses employ the Hatch-Slack cycle and are C4 (Scholes & Walker, 1993; Lajtha & Michener, 1994). C3 and C4 plants discriminate disproportionately against the heavier  $^{13}\text{C}$  isotope and thus bear different isotopic signatures, which can be used to differentiate their relative contribution to root phytomass with the aid of a mixing model (Ludlow *et al.*, 1976; Svejcar & Boutton, 1985; Polley *et al.*, 1992; Dawson *et al.*, 2002).

Stable carbon isotope composition in rooting depth studies has frequently been derived by means of root material (e.g. Ludlow *et al.*, 1976; Svejcar & Boutton, 1985; Polley *et al.*, 1992; Le Roux *et al.*, 1995; Mordelet *et al.*, 1997; Eleki, 2005), and soil organic carbon (e.g. McPherson *et al.*, 1993; McClaran & McPherson, 1995). One area which has received good attention is the humid Lamto savanna in East Africa. Here both oxygen (Le Roux *et al.*, 1995) and carbon (Le Roux *et al.*, 1995; Mordelet *et al.*, 1997) isotopes have been used to investigate spatial partitioning of soil water and rooting patterns between the two savanna growth forms. Both studies indicated that no spatial partitioning exists between trees and grasses; both competing for soil water in the upper 20 cm of the soil. An explanation for this co-occurrence might be that humid savannas (average rainfall of 1200 mm) are nutrient limited, rather than water limited (Le Roux *et al.*, 1995).

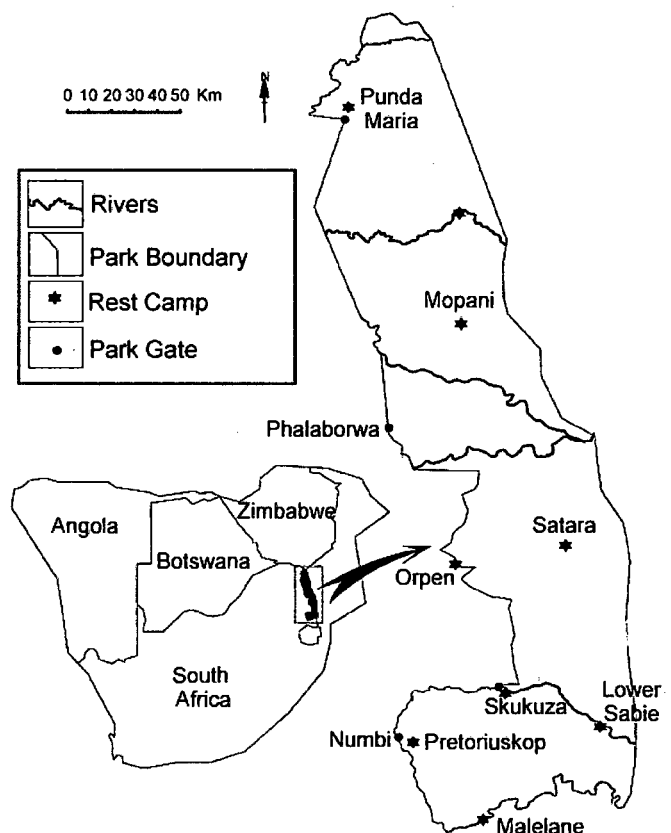
Broadly, this study aims to assess the applicability of Walter's (1971) rooting niche hypothesis in the context of a southern African mesic savanna. Stable isotopes of carbon will be used to describe the rooting profiles of trees and grasses both under tree canopies and in the open. The utility of nitrogen isotopes will be tested with respect to potential

nutrient gradients down the profile. Lastly, information will be synthesized to determine if this mesic savanna is nutrient or water limited.

## Methods

This study was performed in Kruger National Park (KNP), situated in the north-eastern corner of South Africa, bordered by Mpumalanga Province to the south, Limpopo Province to the west, and the Mozambique to the east. KNP is about 19 000 km<sup>2</sup> in size and lies within a mesic region, experiencing hot humid summers and mild, dry winters. The average daily summer temperature is 26.3°C, while the winter is somewhat colder at 17.5°C (Zambatis, 2003). Rainfall is a summer phenomenon, usually falling between October and March as a result of convectional thunder-storms. These occur due to the influx of hot moist air from the tropics in response to anticyclonic activity. Total annual precipitation is thus highly variable across the park, differing by over 300 mm from south to the north. Pretoriuskop receives an average of 746 mm of rain annually (Zambatis, 2003).

The study was carried out in the Pretoriuskop research exclosure (31.14°05" E, 25.08°43" S), approximately 50 km south-west of the main Skukuza Rest Camp, situated in the south of the park. The exclosure is maintained by the Tree-Grass Programme, run by the Botany Department at the University of Cape Town for the last four years. The 7 ha site is



**Fig. 1.** Geographic location of Kruger National Park, within South Africa. The study site is north west of Pretoriuskop Restcamp (Adapted from Harrington *et al.*, 1999).

surrounded by a 2.8 m high, 9000 volt electrified fence (L. Swemmer, pers. comm.), ensuring the exclusion of all large mammals. The site is also encircled by a fire break, and thus has not been burnt for four years.

The Pretoriuskop site is underlain by the Nelspruit Granite Suite comprised of migmatite, gneiss and granite (Barton *et al*, 1986). The associated soils are usually deep (>100 cm) coarse sands, which are eutrophic, apedal and red in colour (Venter, 1986). However, hardpan ferricrete and clayey subsoils are often associated with hydromorphy along midslopes (Venter, 1986). Vegetation is characteristically open tree savanna with dense grass cover and typical savanna elements such as *Terminalia sericea* and *Dichrostachys cinerea* as well as *Hyperthelia dissoluta* and *Setaria sphacelata* (Venter, 1986).

Data was collected in early June, 2006, over a period of two weeks. Five 'open' and five 'closed' sites were selected within the enclosure, based on canopy shading. Open sites were selected for distance away from large trees, and generally in areas exposed to direct sunlight for the majority of the day. Closed sites were chosen beneath large tree canopies, preventing direct sunlight from reaching the site for a large portion of the day.

Species and relative abundance information was collected for tree and grass species within a 2 m radius of the sampling sites. The tallest 10 trees within a 10 m radius of the site were identified and height and basal stem diameter measured.

A 1.5 m deep hole was dug at each of the ten sites and 20×20 cm soil samples extracted from a smoothed vertical profile. Care was taken not to sample beneath grass clumps, as these could potentially have biased the sample (Mordelet *et al*, 1997). Sampling occurred at 5 cm intervals for the first 20 cm, after which sampling occurred every 20 cm up to a depth of 140 cm. z ?

Roots were separated from the soil samples by initial sieving through an 850  $\mu\text{m}$  mesh sieve. The sieved soil was then scrutinized for any root material which might have passed through the sieve, and this was removed manually. Roots were washed to remove all inorganic particles, and dried at 70°C until constant weight was achieved.

Similar to findings in a west African savanna, it was not possible to distinguish grass and tree roots less than 2 mm in diameter (Mordelet *et al*, 1997). Roots were therefore separated into greater and less than 2 mm groups in order to obviate a large tree root bias during mass spectrometer analysis. Large and fine root groupings were weighted and root density calculated for all depths at each site.

In preparation for mass spectrometer analysis, the fine root material (<2 mm diameter) was ground into a homogenous powder form, first using a Wiley mill and then a Retsch MM200 ball-bearing mill.

The  $^{13}\text{C}$  stable isotope approach was used to determine the ratio of a mixed fine tree/grass root material at increasing depth. This is possible because South African savannas are typically comprised of C3 trees and C4 grasses (Vogel, 1978). The ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  differs between plants with different photosynthetic pathways and this can be used to quantify the relative contribution of C3 and C4 plants to a mixed sample (Ludlow *et al*, 1976). C3 species discriminate unequally against the heavier  $^{13}\text{C}$  isotope during the fractionation process and thus contain approximately 14‰ less  $^{13}\text{C}$  than C4 plants (Svejcar & Boutton, 1985).

A Finnegan Matt 252 light isotope radio mass spectrometer was used to measure the stable carbon isotopic ratio in the samples, relative to the PDB international standard, yielding a value for the stable isotope composition of the sample, expressed as  $\delta^{13}\text{C}$ . The PDB standard originates from the fossil skeleton of

*Belemnitella americana* in the Peedee formation, Carolina, USA, and is measured at a  $^{13}\text{C}/^{12}\text{C}$  ratio of precisely 0.0112372 (Craig, 1957). The formula for calculating the stable isotopic composition is:

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where  $R = ^{13}\text{C}/^{12}\text{C}$  and  $\delta^{13}\text{C}$  is expressed in ‰.

Negative  $\delta^{13}\text{C}$  values indicate that samples are depleted of  $^{13}\text{C}$  relative to the PDB standard (Eleki *et al*, 2005). As a result of unequal fractionation,  $\delta^{13}\text{C}$  values are thus more negative for C3 plants and less so for C4 plants, resulting in the two photosynthetic systems grouping discretely. This has proved very useful in tracing a number of ecological processes (Svejcar & Boutton, 1985).

To determine the relative proportion of C3 (tree) and C4 (grass) root material contributed to a mixed sample, the most abundant tree and grass species was collected from within a 2 m radius surrounding each study plot and the  $\delta^{13}\text{C}$  value determined for each. This yielded a mean representative  $\delta^{13}\text{C}$  value of -27.61‰ for trees and -13.26‰ for grasses at the study site (similar to  $\delta^{13}\text{C}$  values for the humid Lamto savanna (Lepage *et al*, 1993)). The isotopic composition of each mixed root sample depends both on the mean representative isotopic composition of tree and grass, as well as their relative proportions in the root sample (Ludlow *et al*, 1976; Svejcar & Boutton, 1985; Polley *et al*, 1992; Lepage *et al*, 1993; Dawson *et al*, 2002). This gives

$$[1] \quad (-27.61)t + (-13.26)g = \delta_s$$

where -27.61 is the representative mean  $\delta^{13}\text{C}$  for C3 plants (i.e. trees) within the study site;  $t$  is the proportional contribution of fine tree roots to the sample; -13.26 is the representative mean  $\delta^{13}\text{C}$  for C4 plants (i.e. grasses) within the study site;  $g$  is the proportional contribution of fine grass roots to the sample; and  $\delta_s$  is

the observed  $\delta^{13}\text{C}$  value for the sample. As  $t + g = 1$ , we have (for tree root material):

$$[2] \quad (-27.61)t + (-13.26)(1-t) = \delta_s$$

The fractional contribution of tree root material to the sample can thus be calculated from the function:

$$[3] \quad t = (\delta_s - (-13.26))/((-27.61) - (-13.26))$$

Naturally, calculating the proportion of grass to the sample density can either be done by switching  $t$  and  $1-t$  in step [2] for  $1-g$  and  $g$ , respectively, or by subtracting  $t$  from 1. Finally, the proportional value for  $t$  and  $g$  are multiplied by the sample density to calculate the respective contributions of trees and grasses to the sample root density.

The representative mean  $\delta^{13}\text{C}$  value calculated from trees and grasses surrounding the study plots has a large bearing on the final calculated contribution of tree and grass the sample (Lepage *et al*, 1993). These values are believed to be accurate, as trees within the study site are all C3 and grasses, C4. Moreover, the standard deviation for 13 representative tree species was 2.17 and for 10 grass species it was 0.86, indication little variation about the mean values.

Using the same root samples as for carbon isotope analysis, relative abundances of  $^{15}\text{N}$  and  $^{14}\text{N}$  isotopes were determined by means of the Dumas combustion method (Shearer & Kohl, 1993) and expressed as  $\delta^{15}\text{N}$  in ‰:

$$\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where  $R = ^{15}\text{N}/^{14}\text{N}$ .

$\delta^{15}\text{N}$  values are expressed relative to atmospheric  $\text{N}_2$ , which is defined as having a  $\delta$ -value of zero (Handley & Raven, 1992). A greater loss of  $^{14}\text{N}$  relative to  $^{15}\text{N}$  (due to, for example, particulate N decomposition in soils) would yield more positive values of  $\delta^{15}\text{N}$  (Peterson & Fry, 1987), while the converse would be true for losses of  $^{15}\text{N}$ .

Data were analysed and interpreted with the aid of Microsoft Excel and Statistica 7 software package. T-tests for independent variables were used to assess whether there were significant differences in fine root density between treatments (i.e. open sites vs. canopy sites) and between grass and tree components within and between treatments.

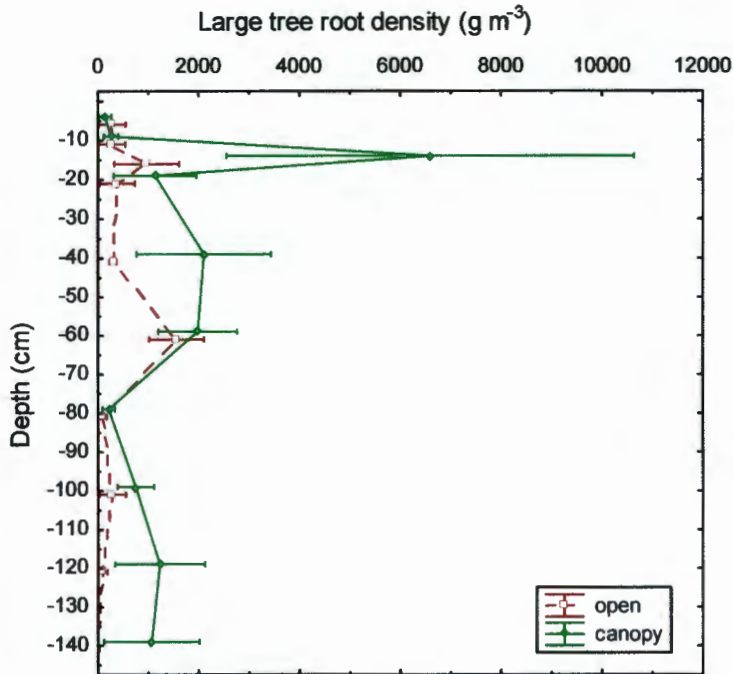
# Results

## Soil characteristics

Soils were generally a reddish colour except for the upper 15-20 cm, which was often dark brown and rich in organic sediments. Soil texture ranged from fine to medium grained sand in the upper horizons, while a gravelly ferricrete layer was encountered towards the bottom of the profile.

## Spatial distribution

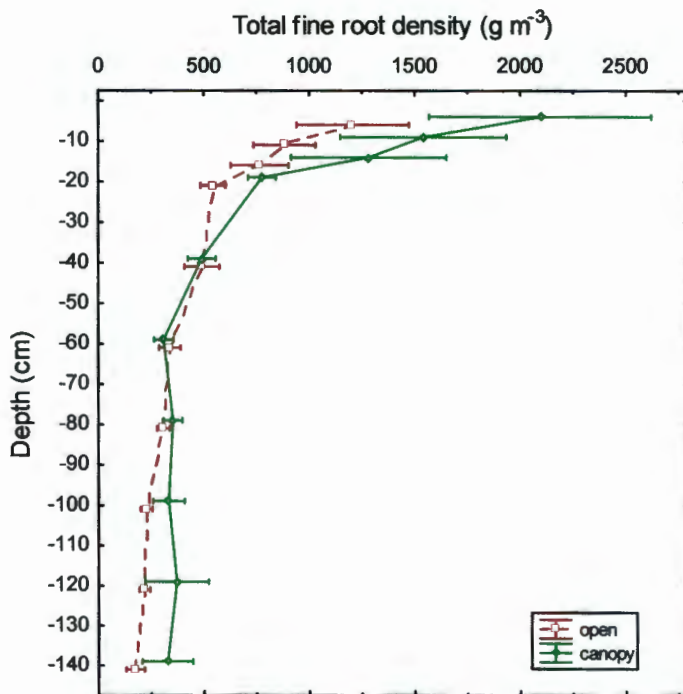
Significance was determined between treatments as a whole. It was found that total fine root density was significantly different between open and canopy treatments ( $t = -2.13$ ;  $df = 98$ ;  $P = 0.04$ ). However, there was no significance between natural abundance



**Fig. 2.** Vertical distribution of large tree root (>2 mm) density beneath the canopy and in the open, with associated standard errors ( $n = 5$ ).

measures of grass density at open and canopy sites ( $t = -0.60$ ;  $df = 98$ ;  $P = 0.55$ ). On the contrary, natural abundance measures of tree density at open and canopy sites was highly significant ( $t = -2.60$ ;  $df = 98$ ;  $P = 0.01$ ).

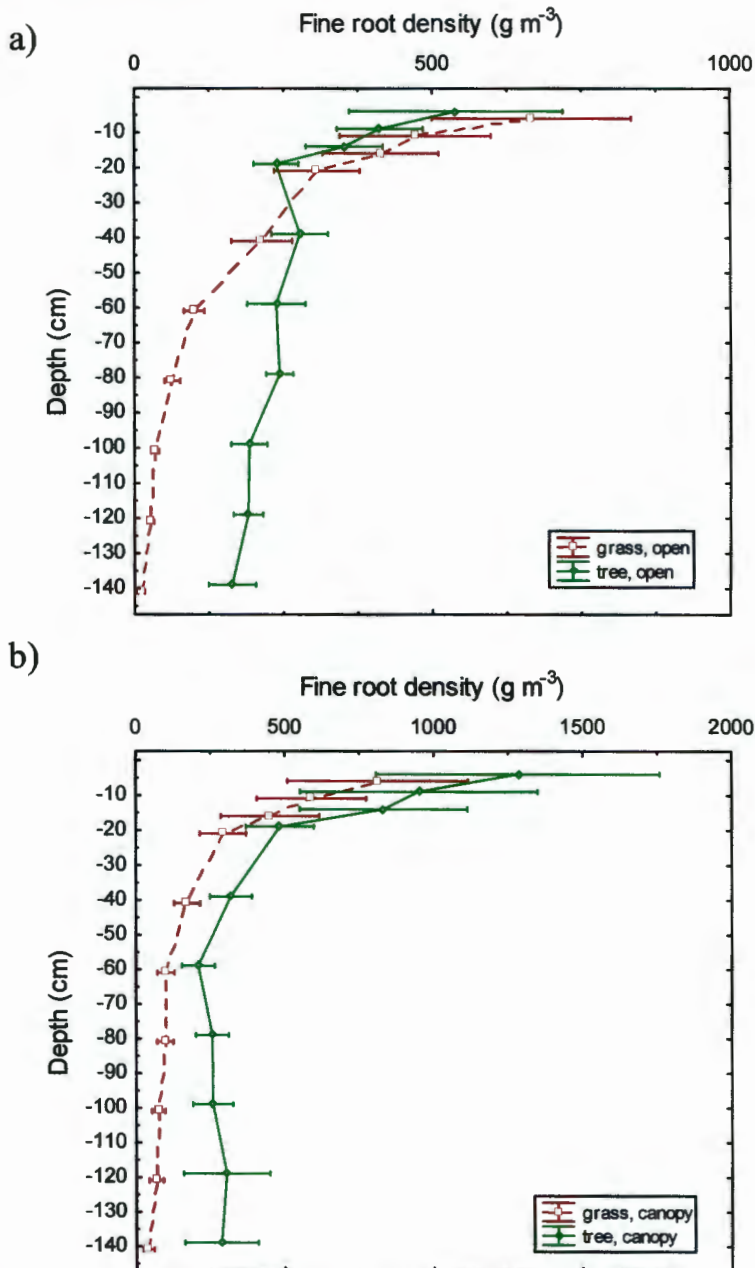
Large tree root (>2 mm) density was highly variable and displayed large standard error with respect to canopy sites (Fig. 2). An example of this can be seen in the 10-15 cm depth class under the canopy treatment (Fig. 2). When all sites within a treatment were combined, large roots under canopy sites had a significantly higher density compared to open sites ( $t = -2.14$ ;  $df = 98$ ;  $P = 0.03$ ).



**Fig. 3.** Vertical distribution of total fine (<2 mm) root density beneath the canopy and in the open, with associated standard errors ( $n = 5$ ).

Mean total (tree + grass) fine root density was higher than mean large tree root density for the open treatment, but not for the canopy treatment. Total fine root density decreased with increasing depth at both open and closed sites (Fig. 3). However, the only significant difference in total fine root density between the two treatments was at the 15-

20 cm soil horizon ( $t = -2.47$ ;  $df = 8$ ;  $P = 0.04$ ). Uncertainty was higher in canopy sites, especially in the upper 15 cm. Total fine root density decreased rapidly between 0-20 cm and then changed little for the remainder of the soil profile.

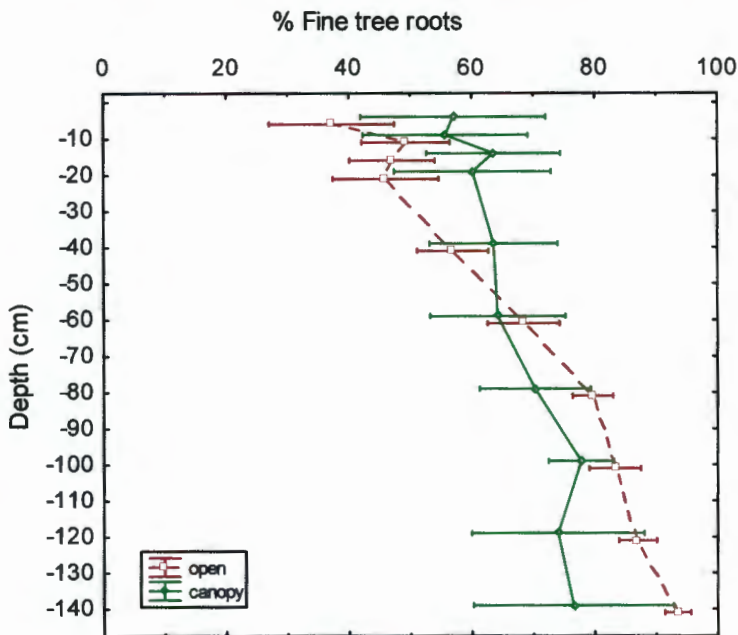


**Fig. 4.** Vertical distribution of fine root (<2 mm) density for a) sites in open savanna, and b) sites beneath the canopy, with associated standard errors ( $n = 5$ ), using  $\delta^{13}\text{C}$  natural abundance technique.

Canopy sites consistently had a higher (initially almost double) density of fine root material, with the exception of the 40-60 cm depth class, where there was a shift in dominance.

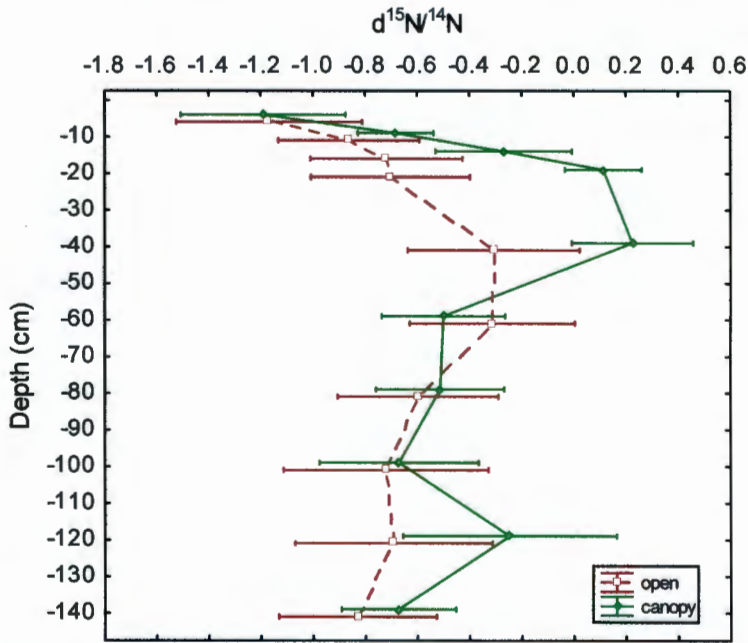
Both tree and grass fine roots decreased with increasing depth in open sites (Fig. 4a). Grass fine roots had a higher density compared to tree fine roots for the initial 30 cm of soil depth. Below 30 cm the relative dominance switched to tree roots, which were significantly more abundant for the remainder of the profile (range:  $t = -2.52$ ;  $df = 8$ ;  $P = 0.04$  to  $t = -6.50$ ;  $df = 8$ ;  $P = 0.00$ ). Uncertainty was higher within the upper soil horizons (0-20 cm) for both treatments. However, the decline was smoother for grasses, while tree fine roots decreased rapidly in the initial 20 cm and little after that.

Sites beneath the canopy indicated a gradual decline in fine root density for both trees and grasses (Fig. 4b). However, contrary to open sites, tree fine roots dominated throughout the profile. However, this difference was only significant between 60-100 cm ( $t = -2.45$ ;  $df = 8$ ;  $P = 0.04$  for both 60-80 and 80-100 cm). Again, uncertainty was high in the upper 20 cm, and remained slightly higher for tree roots throughout.



**Fig. 5.** Fine tree root (<2 mm) density beneath the canopy and in the open savanna, calculated as a percentage of the total fine root density with the aid of natural abundance measures. Associated standard errors are attached ( $n = 5$ ).

The percentage of fine tree roots displayed less of a range in densities but more uncertainty when compared between canopy and open sites (Fig. 5). In the canopy treatment, fine tree roots constituted 55 % of total rooting material at the top of the profile, and 77 % at the bottom. In contrast, open sites were made up of 38 % tree fine root material at the top, and 94 % at the bottom.



**Fig. 6.** Change in  $\delta^{15}\text{N}/^{14}\text{N}$  ratio for fine root (<2 mm) material with increasing depth, in canopy sites and open savanna. Associated standard errors are attached ( $n = 5$ ).

There is a general increase in proportional abundance of  $^{15}\text{N}$  between 0-40 cm after which canopy  $^{15}\text{N}/^{14}\text{N}$  becomes more variable compared to open  $^{15}\text{N}/^{14}\text{N}$  (Fig. 6). The initial increase is greater in canopy sites. In both instances the uncertainty remains high throughout the soil profile, although open sites display less variability.

## Discussion

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The general trend in spatial distribution of fine root density described in this paper is in agreement with many previous studies into tree-grass rooting depths in savannas where moderate to high levels of rainfall occur as a summer phenomenon (e.g. Belsky, 1994; Le Roux *et al.*, 1995; Mordelet *et al.*, 1997). Total (tree-grass mixture) fine root density for both open and canopy covered sites was highest in the upper 20-30 cm of the soil profile and extended to a depth of 140 cm where they were considerably less dense, but still present (see Fig. 3). This provides evidence that there is strong competition for resources in the upper soil horizons. The marked decrease in fine root density with depth illustrated in Fig. 3 provides evidence of strong competition for resources in the upper soil horizons. Whether this competition is for water or nutrients is not clear, although classification of the soils as eutrophic (Venter, 1986) may advance the case of the former, as this would discount nutrients as limiting.

The high variability and uncertainty within the large tree root component was chiefly due to bias caused by large roots passing through some layers and not others (see Fig. 2), but also the result of definite stratification at lower levels of the soil profile. According to Mordelet *et al.* (1997), large root density is probably influenced more by architectural determinants such as soil structure, while fine root density is more likely to be controlled by environmental factors such as soil nutrient and water availability. Good evidence for this statement is provided by a sudden decrease in large tree root phytomass below 80 cm, presumably as a result of the ferricrete layer which is considerably more



Fig. 7. A mixed soil and nodular ferricrete conglomerate found consistently below a depth of 80 cm at the Pretoriuskop study site.

gravelly and compact than overlying sandy sediments and hence more difficult for roots to penetrate (Fig. 7). Total fine root density seems less affected, continuing a gradual decline towards the bottom of the profile. A similar ferruginous layer was also present in the humid Lamto savanna in east Africa, although its effect on large and fine root density distribution was not discussed (Menaut & Cesar, 1979; Mordelet *et al*, 1997).

The significantly greater density of total fine roots and large tree roots in canopy covered sites (see Fig. 2 and 3) indicate that there is some amelioration of climatic stressors and/or increased nutrient availability under trees (Le Roux *et al*, 1995; Mordelet *et al*, 1997). This assertion is supported in certain studies (e.g. Weltzin & Coughenour, 1990; Vetaas, 1992; Belsky, 1994) and is contradicted others (e.g. Mordelet, 1993; Mordelet & Menaut, 1995). The reason for inconsistency may be climatic in origin. Studies which have shown a decrease in understorey productivity have been located in extremely high rainfall, humid savannas (e.g.  $\pm 1200$  mm for the Lamto savanna (Mordelet *et al*, 1997)). Because of a plentiful supply of water, which in turn mobilizes more nutrients, tree canopies were more dense and allowed only 15% of photosynthetically active radiation (PAR) to reach the understorey (Mordelet, 1993). This contrasts strongly with studies in more water limited savanna environments, where transmittance of PAR is approximately 60% (Mordelet, 1993).

No data were available, but one can reasonably infer that trees within the mesic moisture regime of southern Africa - a mixture between broad and fine leaved varieties (van Wyk & van Wyk, 1997) - are characterized by a transmittance of at least 50%. This would allow intermediate levels of shading, which would reduce understorey temperatures and irradiance and consequently provide an environment characterized by decreased plant water stress (Weltzin & Coughenour, 1990; Vetaas, 1992). Additionally, trees contribute to the understorey nutrient pool through litter and dust inputs, increased moisture from stemflow and increased amounts of mineralizable N due to greater densities of soil microbes (Weltzin & Coughenour, 1990; Belsky, 1994; Scholes & Walker, 1997). Weltzin & Coughenour (1990) reported herbaceous productivity of  $260 \pm 17$  g m<sup>-2</sup> at the tree bole, which declined to  $95 \pm 8$  g m<sup>-2</sup> in tree interspaces, highlighting the importance of

trees as nutrient hotspots.

While any interpretation of nitrogen isotopes is speculative because of the disparate sources contributing to the  $\delta^{15}\text{N}$  value from the field, nitrogen analysis in the current study partly supports the argument that greater quantities of mineralizable nitrogen are available beneath tree canopies (Weltzin & Coughenour, 1990; Belsky, 1994; Scholes & Walker, 1997). This is reflected in an increasingly positive  $\delta^{15}\text{N}$  for the upper 40 cm of the soil profile in canopy covered sites (see Fig. 6). The same trend is evident in open savanna sites, but to a lesser extent.

Soil nitrogen is more abundant in  $^{15}\text{N}$  than atmospheric  $\text{N}_2$ , which suggests that plants in canopy covered sites are preferentially obtaining their N without the aid of microbial symbionts, while plants in the open savanna are employing the opposite strategy. The rapid increase in  $\delta^{15}\text{N}$  from 0-20 cm can be explained by the presence of organically rich material which frequently has a higher  $\delta^{15}\text{N}$  value. General negative trends in the lower half of the soil profile may be as a result of  $^{14}\text{N}$  leaching through the ferricrete layer, thereby increasing the fractionation of  $^{15}\text{N}$ , which consequently makes  $\delta^{15}\text{N}$  more negative.

Results from the natural abundance technique used to determine the relative proportion of fine root material contributed by C3 (trees) and C4 (grasses) (Ludlow *et al*, 1976) clearly indicate that tree and grass roots occur throughout the profile in both treatments (Fig. 4a and b). This is contrary to Walter's (1971) rooting niche hypothesis, in which he states that trees and grasses occupy and exploit different soil horizons. Moreover, both grasses and trees seem to favour the upper 20-30 cm of soil, compounding the evidence that resources are disproportionately distributed in this area.

Despite higher rainfall, humid savannas compared well with mesic savannas with respect to general trends in spatial root partitioning in the upper 60 cm (e.g. Mordelet *et al*, 1997). This was especially true beneath canopy sites, where tree and grass fine root densities were only  $\pm 100 \text{ g m}^{-3}$  higher in humid, canopy covered sites when compared to

mesic sites (see Fig. 4b). A significant difference in mean tree fine root density between canopy and open treatments serves to confirm an earlier argument for improved soil moisture and nutrient concentrations under the canopy. The increased moisture and nutrient status would both be able to support a greater biomass, as well as fuel fierce competition between trees and grasses for these nutrients (Weltzin & Coughenour, 1990; Vetaas, 1992; Belsky, 1994). Furthermore, higher soil organic matter and improved water supply through stemflow would not promote root foraging beyond the canopy where nutrient concentrations would be comparatively poor. This is borne out in the data, which indicates that open sites have less than half the density of tree fine roots in canopy covered sites (see Fig. 4a).

The open savanna treatment provided the most interesting insights into mechanisms controlling grass and tree root abundance at Pretoriuskop. While the density of grass fine roots was  $280 \text{ g m}^{-3}$  less in open compared to canopy covered sites, the significant decrease in tree fine roots meant that grass was the initial dominant growth form in the upper soil horizon (see Fig. 4a). However, density dominance switched to trees at about 30 cm below the surface and was significantly different for the remainder of the profile. The lack of significant difference between open and canopy treatments indicate that grasses don't benefit to the same degree as trees do from amelioration of harsh conditions under the canopy. Some small advantage is gained however, as demonstrated by the higher herbaceous biomass found under trees.

Tree fine root density, normally constrained by the presence of grasses, was unusually elevated in the upper 20 cm of the open savanna (see Fig 4a). This can be seen by a sudden deviation in tree fine root density, contrasting strongly with the other root density profiles from this study, which are smoother in appearance. In addition, other studies which have use the same methods for calculating and representing density have not shown this trend and have ended on far lower densities for tree fine roots (e.g. Knoop & Walker, 1985; Le Roux *et al*, 1995; Mordelet *et al*, 1997). For example, in the humid Lamto savanna in east Africa tree fine root density of approximately  $250 \text{ g m}^{-3}$  was

recorded for the open treatment (Mordelet *et al*, 1997), while all other density values were higher when compared to this study.

The increase in fine tree root density could be attributed to the absence of fire and herbivory pressure in the exclosure, which would normally act to keep seedlings and saplings within the flame-zone (Scholes & Archer, 1997). Fire and herbivory have been excluded for over 4 years, while savanna vegetation surrounding the exclosure is burnt approximately every 2-3 years (van Wilgen *et al*, 2000). This suggests that trees surviving in the soil as seedlings and saplings, once freed of the constraints of fire and herbivory, are able to out-compete grasses. This is supported by field observations which noted a definite increase in the number of saplings, especially *Catunaregam spinosa*, *Terminalia sericea* and *Dichrostachys cinerea* in the exclosure, compared to areas outside of the exclosure.

Thus, what might be occurring in the exclosure - due to the exclusion of fire and herbivory - is a gradual shift from a mixed grass and tree savanna, to one dominated by woody elements. Sankaran (2005) suggests that above a mean annual precipitation (MAP) of ~650 mm savanna makes it an inherently 'unstable' system, tending toward a woodland but being held back by disturbances such as fire and herbivory which continually 'reset' the system to a previous state. Pretoriuskop may well represent an example of his theory in practice.

If these predictions are true, it is likely that there will be an increase in the percentage of tree fine roots in the open savanna over time (see Fig. 5). At present tree fine roots only account for 45% of fine roots found in the top 20 cm of the soil profile. Conversely tree fine roots in canopy covered sites dominate the entire profile, ranging from 58 to 93%.

### Conclusion

This study has demonstrated that Walter's (1971) vertical root niche hypothesis does not apply in this mesic southern African savanna. Both tree and grass fine roots occurred

throughout the soil profile but were concentrated in the upper 20-30 cm where they both compete for water and nutrient resources. The results have suggested that there is more than one determinant influencing tree-grass rooting depth. Water may have more of a bearing on rooting patterns arid environments (e.g. Sala *et al*, 1989), while nutrients might play a bigger role in humid savannas (Le Roux *et al*, 1995; Mordelet *et al*, 1997). The investigation into nitrogen in this study did not show any trends due to numerous confounding factors in the expression of  $\delta^{15}\text{N}$ . The use of nitrogen isotopes in revealing patterns in rooting depth as well as nutrient acquisition

Vegetation in the Pretoriuskop exclosure appears to be shifting away from a mixed savanna containing trees and grasses to one where trees dominate. The catalyst for this change is probably the removal of constraining factors such as fire and herbivory, which limit tree seedling/sapling recruitment and establishment. In addition to these factors, this area can be described as 'unstable' according to the rainfall criteria laid out by Sankaran (2005), and thus could be gradually regressing toward a closed woodland in which grasses exist only as marginal vegetation. Should this be the case, it leads us to important questions in a world where future changes in precipitation could greatly influence the distribution of savanna ecosystems

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