Using stable water isotopes to determine the depth of water used by different sizes of savanna trees in Kruger National Park

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APPENDIX 1

ABSTRACT

A curious feature of savanna is the coexistence of trees of grasses. Many studies have attempted to explain this coexistence in terms of root niche differentiation between grasses and trees or as a result of fire frequency. So far little work has been done to investigate whether different size classes of trees are sourcing different water. We sampled boreholes, rainwater and two tree species in Kruger National Park. *Terminalia sericea* has a deep tap root that was hypothesized to be able to access rainwater, whilst *A. nigrescens* is shallow-rooted and therefore it was hypothesized not to be able to access groundwater. We found that large *T. sericea* trees (>6.5 m) (δD = -15.2 ± 1.4‰) had an isotopic composition very similar to that of the closest borehole (δD = -16.5 ± 1.0‰) suggesting that large *T. sericea* trees were sourcing groundwater. Medium-sized *T. sericea* trees (2.5 m – 4.0 m) had an isotopic composition (δD = -10.6 ± 3.5‰) that was more similar to that of precipitation (δD = -11.4 ± 4.9‰) indicating a greater reliance on rainwater than large individuals. Small *T. sericea* trees (<1m) had an isotopic composition (δD = -6.9 ± 3.1‰) that was more positive than that of groundwater (δD = -16.5 ± 1.0‰) or rainwater (δD = -11.4 ± 4.9‰) suggesting that they were sourcing shallow soil water that had undergone evaporation. Large *A. nigrescens* individuals (>3 m) had a isotopic composition (δD = -19.3 ± 5.0‰) similar to that of precipitation (δD = -26.4 ± 3.4‰) suggesting that adult *A. nigrescens* trees were mainly sourcing precipitation. Small *A. nigrescens* individuals (<1 m) had an unexpected similar isotopic composition (δD = -25.0 ± 3.7‰) to groundwater (δD = -26.4 ± 3.4‰) suggesting that small individuals had access to groundwater. Thus this study has demonstrated that different size classes of savannas trees are in fact sourcing water from different depths and this depends on the inherent physiology of the tree that allows gullivers or adults to develop a deep root structure. Thus the fact that trees and gullivers are sourcing water from different depths will have to be incorporated into current savanna modes attempting to explain the coexistence of trees and grasses.

INTRODUCTION

Savanna vegetation is prominent both in Africa and South Africa, occupying about 50% and 35% of the land surface respectively. This wide-spread distribution lies in the ability of savannas to cope with an exceptional spectrum of climatic, edaphic and biogeographic conditions. And yet despite this vast extent savanna remains one of the least understood ecosystems (Cole, 1986). The fundamental characteristic of all savannas is the curious coexistence of trees and grasses (Scholes & Archer, 1997; Higgins *et al.*, 2000). A multitude of factors such as water, herbivory, fire, soil texture and nutrients, influence the balance between grasses and trees in savanna (Cole, 1986). Taking into account the complexity of savannas it is exceptional that trees and grasses are able to coexist over such a wide range of environmental variables – this was first referred to as the "Savanna Problem" by Sarmiento (1984).

In savannas rain falls predominantly in summer and water is limited during winter, thus competition for water has been considered to be especially important in determining vegetation structure in savannas. Walter (1974) proposed that the tree-grass interaction was possible because of spatial separation of rooting niches. Thus trees having deeper roots would have sole access to deeper waters and grasses having their root biomass concentrated in the upper soil layers would be superior competitors for shallow soil water. Walker & Noy-Moir (1982) expanded upon the Walter hypothesis by developing an analytical model based on this hypothesis and demonstrated that rooting niche differentiation could allow the stable coexistence of grasses and trees. Evidence supporting water resource partitioning has been reported for South African savannas (Knoop & Walker, 1985), short-grass steppe in Colorado (Dodd *et al.*, 1998), Kenyan savanna (Helsa *et al.*, 1985), and a temperate savanna in Arizona (Weltzin & McPherson, 1997). There are however several recent studies that bring into question whether the Walter hypothesis (in its purest form) can account for the coexistence of grasses and trees (Scholes & Walker, 1993; Le Roux *et al.*, 1995; Midwood *et al.*, 1998; Mordelet *et al.*, 1997; Higgins *et al.*, 2000). Thus many new models are incorporating a multitude of factors that affect the balance between grasses and trees. For example Higgins *et al.* (2000) developed an analytical model which promotes the coexistence of trees and grasses by incorporating fire, its frequency, distribution, variance and intensity, and also including tree resprouting, and the impact of variability of rainfall on seedling recruitment.

A particularly obvious feature of savannas is the frequent fire events. This prevalence of fire within the savanna ecosystem has had large impacts on the life strategies of both grasses and trees. Grasses have dealt with the 'fire problem' by having very short life cycles, reaching reproductive maturity and senescing within a year. Grasses are killed by a fire but are able to quickly colonise an area. Conversely most tree species are much longer-lived that grasses, typically taking a number of years to reach reproductive maturity. Adult trees are able to survive fires by developing thick bark to protect the living part of the tree and having a large proportion of their foliage out of the height reached by fire (typically about 3 m). Most savanna tree species have seedlings that will first store a large proportion of nutrients in an underground lignotuber before attempting to escape the 'fire-trap' (the height typically reached by fires) (Scholes & Walker, 1993). Thus if they are burnt in a fire before they manage to escape the fire-trap they are able to use the resources stored in the lignotuber to resprout after the fire. During this stage of the tree's life cycle it is called a "gulliver". Thus fire is considered to be a major controlling factor on tree recruitment and thus the balance of trees and grasses (Jeltsch *et* al., 2000; Higgins *et al.*, 2000). Whilst many studies have examined the possibility that grasses and trees are sourcing different from different depths (e.g. Helsa *et al.*, 1985, Weltzin & McPherson, 1997), there have been little work done on investigating the possibility that immature trees may be sourcing different water to large adult trees. This could contribute to the coexistence of trees and grasses by limiting the establishment of gullivers to trees.

Isotopes are variations of an element that have different numbers of neutrons, and therefore differing atomic weights. The difference in mass tends to be more pronounced in the lighter elements such as oxygen and hydrogen (Redwine & Howell, 2002). Isotopic fractionation is the change in isotopic ratios of a material owing to different rates at which various isotopes undergo kinetic and chemical reactions (Dansgaard, 1964). Plants do not fractionate oxygen or hydrogen isotopes during water uptake (Dawson & Ehleringer, 1991), and thus hydrogen or oxygen isotopes in xylem water of plants should reflect those of the water source used by the plant. When water evaporates there is preferential evaporation of lighter isotopes and thus the water left behind is enriched with heavy isotopes. Water that has undergone evaporation has typically more positive δD and $\delta^{18}O$ values than water that has undergone little or no evaporation.

Water found naturally below the water table (i.e. the permanently saturated zone) is normally referred to as groundwater, whilst water that occurs above the water table is usually referred to as soil water. Within the soil profile there is a gradient in isotopic composition (the ratio of heavy to light isotopes) that exists, with soil water in the upper layers typically tending to have more positive δD and δ^{18}O values than water in deeper layers (Ehleringer *et al.*, 1991; Ehleringer & Dawson, 1992; Dawson, 1996). Variations in the isotopic composition within soils can arise because of selective recharge during periods of heavy rainfall (Mazor & Verhagen, 1983), differences in the seasonal input of moisture, and most importantly evaporation of water in the uppermost soil layers (Ehleringer & Dawson, 1992). Shallow soil water undergoes more evaporation than water deeper in the soil profile (or groundwater), and is thus enriched with heavy isotopes (and therefore has more positive δD and δ^{18}O values) relative to water in deeper soil layers (Allison *et al.*, 1983). Thus analysis of stable water isotopes provides a technique to assess and test the spatial utilization (i.e. depth) of water used by plants.

It was hypothesized that larger trees would have the ability to develop roots which are able to access groundwater (i.e their roots are able to reach the permanently saturated zone). However we hypothesized that smaller individuals (i.e. gullivers) and shallow rooted trees did not have the ability to develop deep root structures to access groundwater and thus were restricted to using soil water and rainwater. Because a gradient of isotopic composition exists within the soil profile, it is possible to use hydrogen and oxygen isotopes to test the hypothesis that different sized trees are utilizing water from different depths in the soil profile. Thus we predicted that the isotopic composition of larger trees would be more negative than the isotopic composition of smaller trees and shallow-rooted trees. Thus this study aimed to use oxygen and hydrogen isotopes of xylem water extracted from twigs to establish whether different size classes of trees are utilizing water from different depths. Furthermore we hypothesized that groundwater stable in terms its isotopic composition and was also uniform at each study site. We aimed to test this by collecting groundwater from several boreholes at each site, over a period of months, and analyzing the isotopic composition.

METHODS

Study Area

The Kruger National Park (KNP) (22°25' and 25°32' East and 30°50' and 32° South) is situated in the Lowveld of the eastern parts of the Northern and Mpumalanga provinces of South Africa. The park was established in 1926 and is the largest game reserve in South Africa, conserving almost 20 000 km^2 of land. Topography is mostly flat bush plains and savanna woodlands, with occasional rocky outcrops. Soils can range from granitic origin to basalt plains as well as sandstone and ecca-shales. The climate is subtropical with maximum daily temperatures ranging between 26°C and 33°C and most of the rain falling over the summer months (September to March), with very little rain over the winter months (May to August) (Figure 1). Mean annual rainfall varies from about 750mm in the south of the reserve to approximately 280 mm in the north, but variations around the mean can be marked from year to year (van Wilgen *et al.*, 2000). This climatic and edaphic heterogeneity allows a wide variety of vegetation types (16 different types) to exist in the KNP, from woodlands to bushveld.

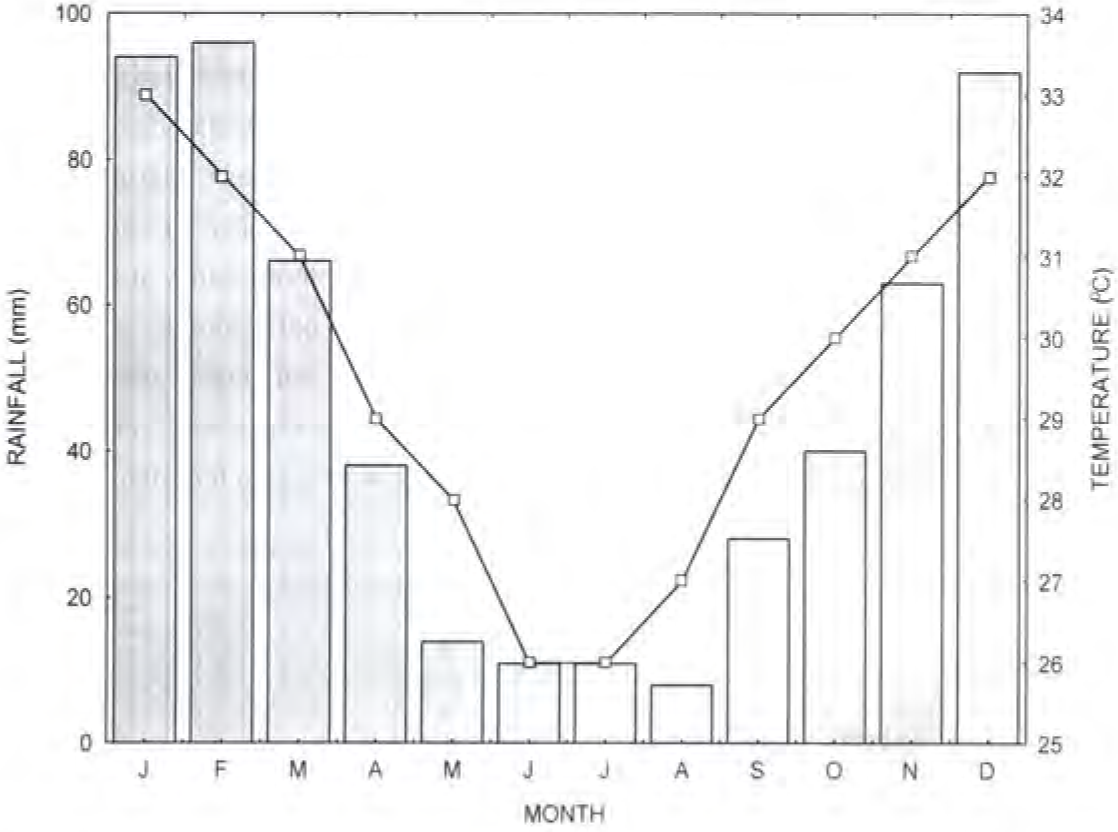

Figure 1: Mean monthly rainfall (bars) and daily maximum temperature (line) for Skukuza (24° 59' S 31° 36' E, 263m elevation) from 1961 to 1990 (Source: South African Weather Bureau).

Within the KNP three study sites were selected, namely Pretoriuskop, Satara and Shingwedzi (Figure 2). These sites are located along a longitudinal rainfall gradient in the reserve with the wettest site located at Pretoriuskop (625-750 mm), followed by Satara (550 mm) and the driest site located in the north at Shingwedzi (375 – 500 mm) (van Wyk, 1974). Groundwater was taken from four boreholes at Pretoiuskop, four boreholes at Satara, and two boreholes at Shingwedzi (Table 1).

Table 1: The names of boreholes sampled at each study site and the depth to the water table at the end of May.

Pretoriuskop		Satara		Shingwedzi
Kirkman	10.4 m	Nwanetsi	6.3 m	Sandpiper
Morester	8.3 m	Ngirivane	6.2 m	Tomlinson
Hlangwini		Sweni 1	4.7 m	
Sithungwane	12.3 m	Sweni 2	4.4 m	

At the Pretoriuskop study site *Terminalia sericea* is the dominant tree. *T. sericea* has an exclusive preference for deep, well-drained soils and it widespread over the granite sections of the park. Soil at this site was deep (> 1m), nutrient-poor and sandy with a dark red colour. Trees were sampled from the Pretoriuskop exclosure (Figure 3). This is an experimental exclosure designed to exclude animals from the site so that the influences of herbivory can be observed. At this particular site adult *T. sericea* trees attained a maximum height of 10 meters (Appendix 1, table A), although mature adult trees can grow up to 20 meters tall. Adults tended to be single stemmed with a multi-tiered, rather flat crown. Gullivers tended to have multiple stems that originated from a single rootstock.

At the Satara site the dominant tree species is *Acacia nigrescens*, which is a remarkable species in that grows on all geological formations within the reserve, but is not usually found in close proximity to a water source (van Wyk, 1974). Soil at this site was shallow (usually <75cm), nutrient rich clay with a dark brown colour. Trees were sampled from the Satara exclosure within the buffalo inclosure (Figure 4). The Satara exclosure is another experimental site to observe the effects on vegetation when herbivores are removed. At this site adult individuals attained a maximum height of 5 meters (Appendix 1, table B), although mature *A. nigrescens* trees can reach a size of 18 meters. Large *A. nigrescens* trees at this site usually had a major branch of the stem close to the soil surface, although some individuals had single stems.

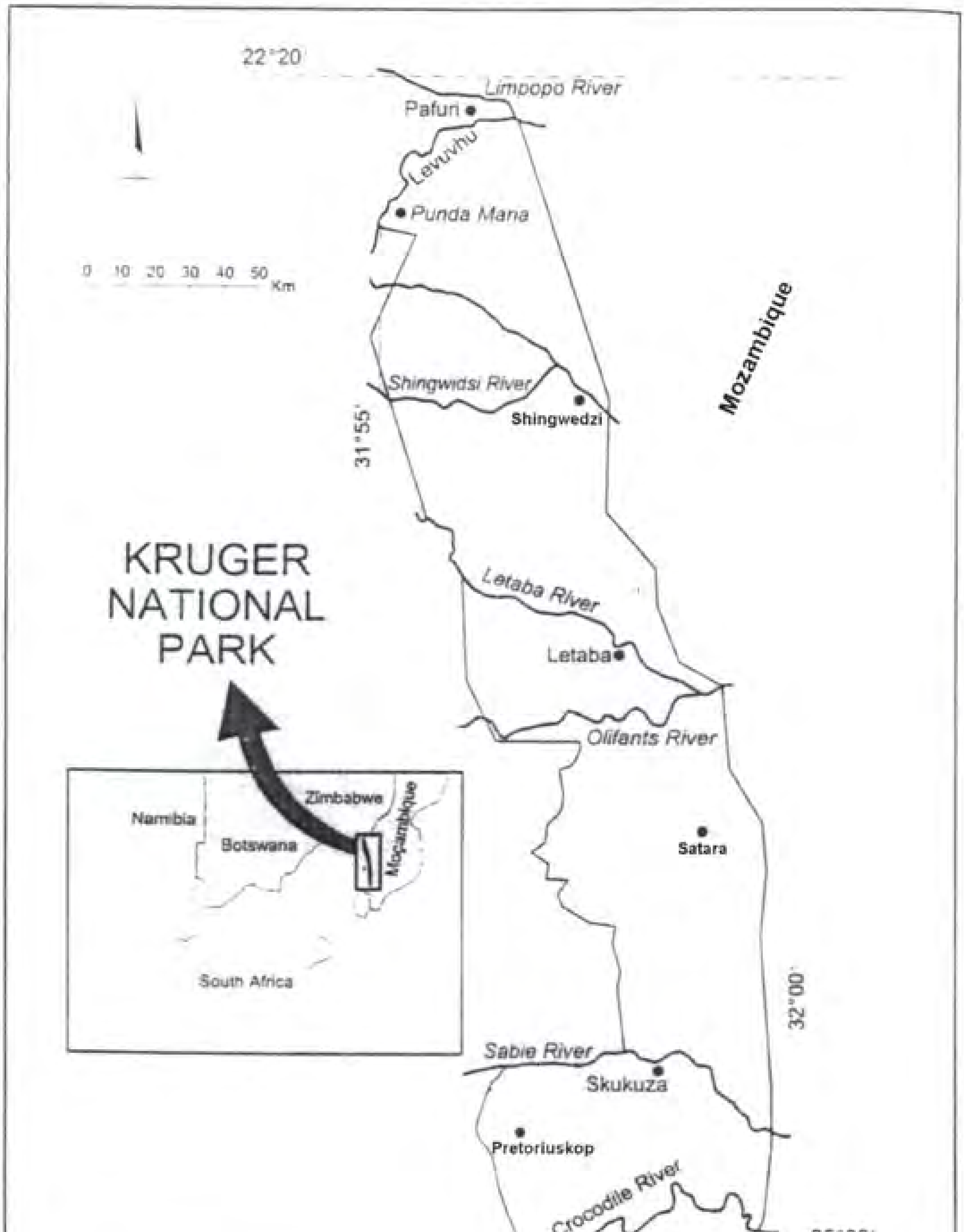

Figure 2: Map of the Kruger National Park showing the locality of the study sites at Satara, Shingwedzi and Pretoriuskop (Source: Adapted from Van Wilgen *et al.*, 2000)

Figure 3: Map showing the locality of the Pretoriuskop exclosure site (tree sampling site) and the locality of the four sampled boreholes, Kirkman, Morester, Hlangwini, and Sithungwane (red dots).

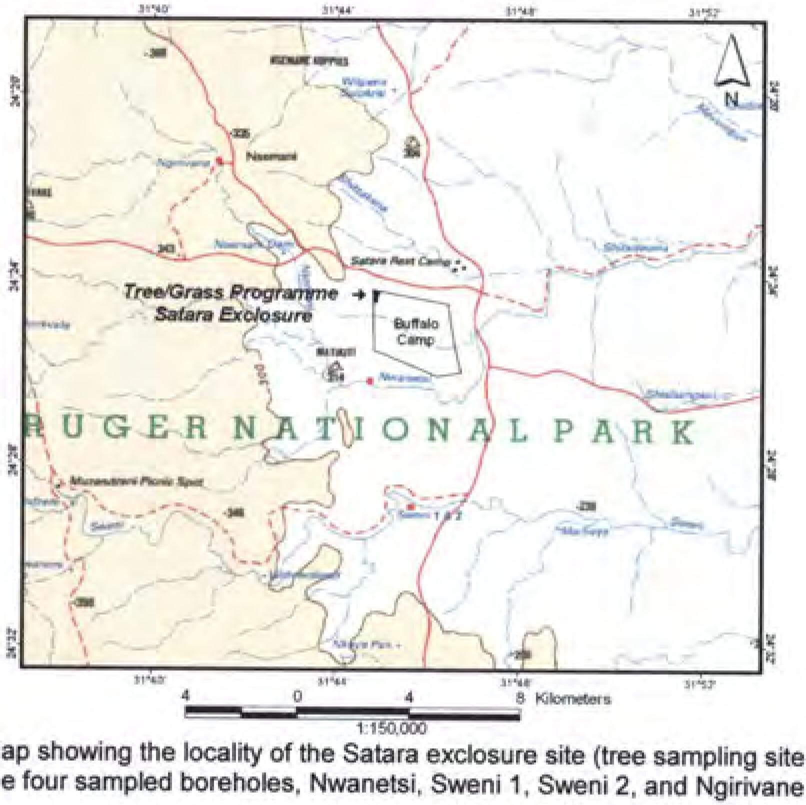

Figure 4: Map showing the locality of the Satara exclosure site (tree sampling site) and the locality of the four sampled boreholes, Nwanetsi, Sweni 1, Sweni 2, and Ngirivane (red dots).

The canopy of large *A. nigrescens* individuals tended to be conical in shape with some branches close to the ground. Gullivers tended to be multi-stemmed with the branches forming an impenetrable 'cage'.

At Pretoriuskop three size classes of *Terminalia sericea* were identified; small trees (small gullivers)(<1 m), medium trees (large gullivers) (2.5 m – 4.0 m), and large trees (adults) (>6.5 m) (Appendix 2). At the Satara site only two size classes were identified; gullivers (<1 m) and trees (> 3 m) (Appendix 2). All size classes were selected so as to avoid any overlap of the size classes within a species. Two twig samples were taken from six individuals of each size class, during June (middle of the dry season). Only non-photosynthetic, suberised twigs were taken as samples because transpiration or evaporation can cause fractionation (Ehleringer & Dawson, 1992). Once cut, twig samples were quickly sealed in a Kimax® tube with the lid sealed shut with Parafilm®. Soil samples from different depths of a soil auger were taken to measure gravimetic water content and to test the possibility of analyzing isotopic composition of soil water. Soil samples were double sealed in plastic packets to prevent evaporation of soil water. Groundwater was collected (mainly) from decommissioned boreholes surrounding the exclosures during the months of April, May. Water from boreholes should reflect the isotopic composition of groundwater, because boreholes penetrate the permanently saturated zone. Rainwater was collected for each month (if it rained) from specially constructed rain gauges. Rain gauges were made from 500 ml jars covered with mesh and a funnel at the top to collect rainwater. Paraffin was placed at the bottom of the jar. Paraffin has a lower density than water and therefore paraffin floats on water. Thus paraffin prevents evaporation of rainwater, and thus enrichment of the water, from the jar. All twig samples were frozen in a deep-freeze, soil water samples placed in a fridge, and groundwater and rainwater samples were stored in Vacutainers® sealed with Parafilm® and placed in a cool cupboard.

Water was extracted from twig samples using cryogenic distillation method (Dawson, 1996). Oxygen isotopes were extracted from the samples using the CO_2 equilibrium method of Socki *et al.* (1992). This method involved prefilling Vacutainers® (7 ml airtight glass vials) with about 500 mbars of medical grade CO_2 and inserting 1 to 1.5 ml of sample water into the prefilled Vacutainers®. The filled Vacutainers® were placed for between 12 and 18 hours in a gently rocking apparatus in a water bath with the temperature set at 25°C. An equilibrium exchange process occurs (2) and the oxygen isotope signature of the sample water becomes reflected in the oxygen of the carbon dioxide gas. The carbon dioxide gas was extracted by connecting the sample to a vacuum line with a series of U-tubes and using liquid nitrogen and an ethanol slush to eliminate non-condensable gases, such as oxygen and nitrogen, from the sample

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad (2)$$

A variation of the zinc closed tube reduction method (Coleman *et al.*, 1982) was used to measure hydrogen isotopes. This method involved introducing approximately 2 µl of water to 105-120 mg of zinc turnings. The tube was attached to the vacuum line, frozen in liquid nitrogen, evacuated and then sealed using a acetylene torch. Samples were placed for 2 hours in a furnace at 500°C to reduce the water to H_2 and oxygen, at which point the oxygen readily combines with zinc to form zinc oxide (3) leaving behind just hydrogen gas.

$$Zn + H_2O \rightarrow ZnO + H_2 \qquad (3)$$

Oxygen and hydrogen isotope ratios were measured using a Finnegan MAT252 isotope ratio mass spectrometer, and values are always compared to an international standard, in this case V-SMOW (Vienna-Standard Mean Ocean Water). The deviation from SMOW is denoted by delta (δ) and calculated as:

$$\delta = (R_{sample}/R_{Standard} - 1) \times 1000 \qquad (1)$$

where R is the ratio of the heavy to light isotope (i.e. $R = {}^{18}O/{}^{16}O$ or D/H), and isotope values are reported as parts per thousand or per mil (‰) deviation from V-SMOW.

The equations of Coplen (1993) were used to convert raw data to the V-SMOW scale. Our own independently analysed internal standards (DML ICE and CTMP for hydrogen, and DISTILLED STANDARD for oxygen) were run to calibrate our results to the international standard, V-SMOW as well as to correct for drift in the reference gas.

Data analysis

Data were statistically analyzed using the computer programme STATISTICA 6. T-tests for independent variables were used to test the hypothesis that different size classes of trees had different $\delta^{18}O$ and δD values of xylem water. We also t-tests to test if there were significant differences in $\delta^{18}O$ and δD values of groundwater at study sites and between study sites.

RESULTS

Although soil from Satara appeared to be drier than soil from Pretoriuskop, it actually had a higher water content than soil from Pretoriuskop (Table 2). At Pretoriuskop the soil had a very low water content, which increased with increasing depth in the profile. Whereas at Satara the soil closest to the surface had a much higher water content than Pretoriuskop, which decreased slightly at a depth of 50 cm from the soil surface. However the deepest soil sample at Satara had the lowest water content at the site.

Table 2: Gravimetric water content of soil from Pretoriuskop and Satara. Soil was dried at 90°C for 48 hours.

Pretoriuskop		**Satara**	
Depth (cm)	*Water content*	*Depth (cm)*	*Water content*
30	3.1%	25	12.3%
60	4.0%	50	13.5%
90	4.3%	75	9.1%

A peculiarity of meteoric water worldwide is the remarkable correlation between $\delta^{18}O$ and δD values Craig (1961). $\delta^{18}O$ and δD isotope values of boreholes and precipitation at Shingwedzi, Satara and Pretoriuskop were plotted against each other to construct a meteoric water line for KNP. This line of best line was calculated as $\delta D = 6.84 * \delta^{18}O + 6.57$ ($r^2 = 0.910$). Because of this extremely close relationship between $\delta^{18}O$ and δD values, we have reported both the $\delta^{18}O$ and δD values of groundwater (table 3) and tree samples (table 4), but have only discussed δD values in the text, in order to simplify the results and discussion.

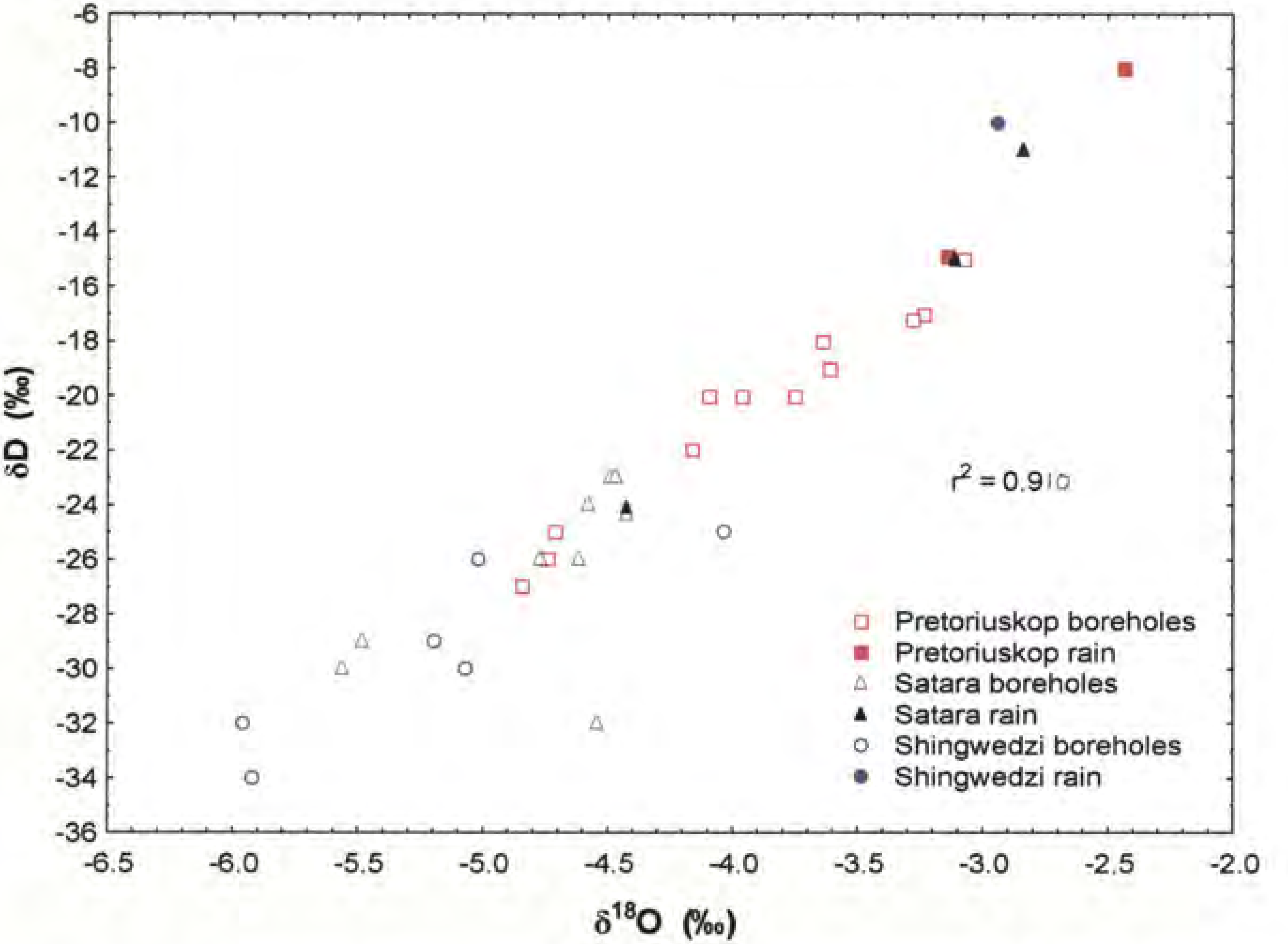

Figure 5: Meteoric water line for boreholes and rainwater at Pretoriuskop, Shingwedzi and Satara, for the months of April, May and June

Groundwater was variable between the three sites with Pretoriuskop having the most positive $\delta^{18}O$ and δD values, and Shingwedzi the most negative, with groundwater at Satara falling in between (Table 3).

Table 3: Mean (and standard deviations of) oxygen and hydrogen isotope ratios of boreholes at Pretoriuskop, Satara, and Shingwedzi over April, May, and June.

Site	*$\delta^{18}O$*	*δD*
Pretoriuskop		
Kirkman	-3.2 ± 0.1‰	-16.5 ± 1.0‰
Morester	-3.7 ± 0.1‰	-18.9 ± 1.1‰
Hlangwini	-4.1 ± 0.1‰	-20.7 ± 1.2‰
Sithungwane	-4.8 ± 0.1‰	-26.1 ± 0.9‰
Satara		
Nwanetsi	-4.6 ± 0.2‰	-24.1 ± 1.4‰
Sweni 1	-4.5 ± 0.1‰	-27.2 ± 6.2‰
Sweni 2	-4.6 ± 0.2‰	-25.0 ± 1.1‰
Ngirivane	-5.6 ± 0.1‰	-29.6 ± 0.9‰
Shingwedzi		
Sanpiper	-4.8 ± 0.6‰	-26.9 ± 4.0‰
Tomlinson	-5.6 ± 0.5‰	-30.5 ± 3.6‰

The very small standard deviations in the hydrogen and oxygen isotope values of groundwater indicate that there was very little variation in the isotopic composition of groundwater at each borehole from April to June. At Pretoriuskop, Kirkman borehole was the closest borehole to the tree-sampling site and this had the most positive groundwater isotope ratio (δD = -16.5 ± 1.0‰). There was progressive decrease of the oxygen and hydrogen isotope ratios with increasing distance of the borehole to the tree-sampling site (i.e. a decrease in oxygen and hydrogen isotope ratios from Kirkman, to Morester, to Hlangwini, to Sithungwe). The same effect was not observed at Satara although boreholes that were close to each other e.g. Nwanetsi, Sweni 1, and Sweni 2 had similar $\delta^{18}O$ and δD values to each other, whilst the Ngirivane, which was far away from all three of these boreholes, had much more negative $\delta^{18}O$ and δD values.

Boreholes at Pretoriuskop (δD = -20.5 ± 3.8‰), Satara (δD = -26.4 ± 3.3‰), and Shingwedzi (δD = -29.3 ± 3.4‰) showed some separation in their hydrogen and oxygen isotope values (Figure 5). Over the course of April, May, and June Pretoriukop boreholes had significantly different hydrogen isotope values to boreholes at Satara ($t = 3.71$, d.f. = 19, $P = 0.001$) and Shingwedzi ($t = 4.79$, d.f. = 16, $P = 0.000$). However there was no significant difference between boreholes at Satara and Shingwedzi in terms of δD values (t = 1.69, d.f = 13, P = 0.115).

Winter is the low rainfall period for KNP and thus there was no precipitation collected during May and June at Shingwedzi and also no precipitation was collected at Pretoriuskop during May. The average isotopic composition of precipitation at the different sites was quite variable during April, May and June. At Pretoriuskop April's rainfall was more depleted in hydrogen isotope ratios (δD = -8 ‰) than June's rainfall (δD = -15 ‰). At Satara April's rainfall was also more depleted in hydrogen isotope ratios (δD = -11 ‰) than either May's rainfall (δD = -15 ‰) or June's rainfall (δD = -25‰). The hydrogen isotope ratio for April rainfall at Shingwedzi (the only month it rained at this site) was -10 ‰. Over the period of the study precipitation tended to be have a more positive isotopic composition relative to groundwater (Figure 5). This isotopic enrichment of rainfall can be explained by the amount effect because when light rainfall events occur, such at during winter months in KNP, oxygen and hydrogen isotope values are typically more positive.

Table 4: Mean oxygen and hydrogen xylem isotope ratios of twig samples from *T. sericea* and *A. nigrescens* for different size classes of trees sampled at the end of May

	$\delta^{18}O$	δD
T. sericea		
Large	-1.7 ‰	-3 ‰
Large	-2.1 ‰	-8 ‰
Large	-2.5 ‰	-12 ‰
Large	-1.8 ‰	-5 ‰
Large	-1.8 ‰	-6 ‰
Medium	-2.8 ‰	-13 ‰
Medium	-2.9 ‰	-13 ‰
Medium	-2.3 ‰	-8 ‰
Medium	-2.3 ‰	-8 ‰
Medium	-3.0 ‰	-15 ‰
Medium	-2.7 ‰	-6 ‰
Small	-3.0 ‰	-14 ‰
Small	-3.4 ‰	-16 ‰
Small	-3.3 ‰	-15 ‰
Small	-3.3 ‰	-17 ‰
Small	-2.9 ‰	-14 ‰
A. nigrescens		
Large	-3.6 ‰	-16 ‰
Large	-3.0 ‰	-17 ‰
Large	-3.6 ‰	-15 ‰
Large	-4.0 ‰	-24 ‰
Large	-4.8 ‰	-27 ‰
Large	-3.5 ‰	-17 ‰

Small	-4.4 ‰	
Small	-4.6 ‰	-25 ‰
Small	-5.2 ‰	-30 ‰
Small	-4.8 ‰	-26 ‰
Small	-5.2 ‰	-24 ‰
Small	-4.1 ‰	-20 ‰

The isotope ratios of water extracted from small, medium and large *T. sericea* trees (Table 4) show different sized individuals were sourcing different water sources (Figure 6). Small trees had the most positive δD isotope ratios (-6.9 ± 3.1‰), followed by medium-sized trees (-10.6 ± 3.5‰), whilst large trees had the most negative δD isotope ratios (δD = -15.2 ± 1.4‰). Although small trees showed no significant difference in δD values for small and medium-sized trees (t = 1.86, d.f. = 9, P = 0.096), large trees had significantly more negative δD isotope ratios than both small trees (t = 5.45, d.f. = 8, P = 0.001) and medium-sized trees (t = 2.77, d.f. = 9, P = 0.022). Large individuals (δD = -15.2 ± 1.4‰) had similar hydrogen isotope ratios to the average isotope ratio of the closest borehole (Kirkaman; δD = -16.5 ± 1.0‰) suggesting that large individuals were sourcing this groundwater. The hydrogen isotope ratio of medium-sized trees (δD = -10.6 ± 3.5‰) was slightly above that of groundwater (δD = -16.5 ± 1.0‰) and closer to the average rainfall isotope value (δD = -11.4 ± 4.9‰) possibly indicating a greater reliance than larger individuals on rainwater. The hydrogen isotope ratios of small trees (δD = -6.9 ± 3.1‰) were more positive than those of rainwater (δD = -11.4 ± 4.9‰) indicating that small trees are sourcing an evaporated water source, possibly rainwater which has undergone evaporation.

The hydrogen isotope ratios of large and small trees of *A. nigrescens* individuals show that small individuals were sourcing more negative water than larger individuals (Figure 7), although the δD values of small (-25.0 ± 3.7‰) and large trees (-19.3 ± 5.0‰) were not significantly different (t = 2.12, d.f. = 9, P = 0.063). Small *A. nigrescens* individuals (δD = -25.0 ± 3.7‰) had similar isotopic composition to that of groundwater (δD = -26.4 ± 3.4‰), whilst most large trees had a similar isotopic composition (δD = -19.3 ± 5.0‰) to the average rainfall (δD = -16.7 ± 6.8‰), although two individuals had similar isotope ratios to those of groundwater (Figure 7)

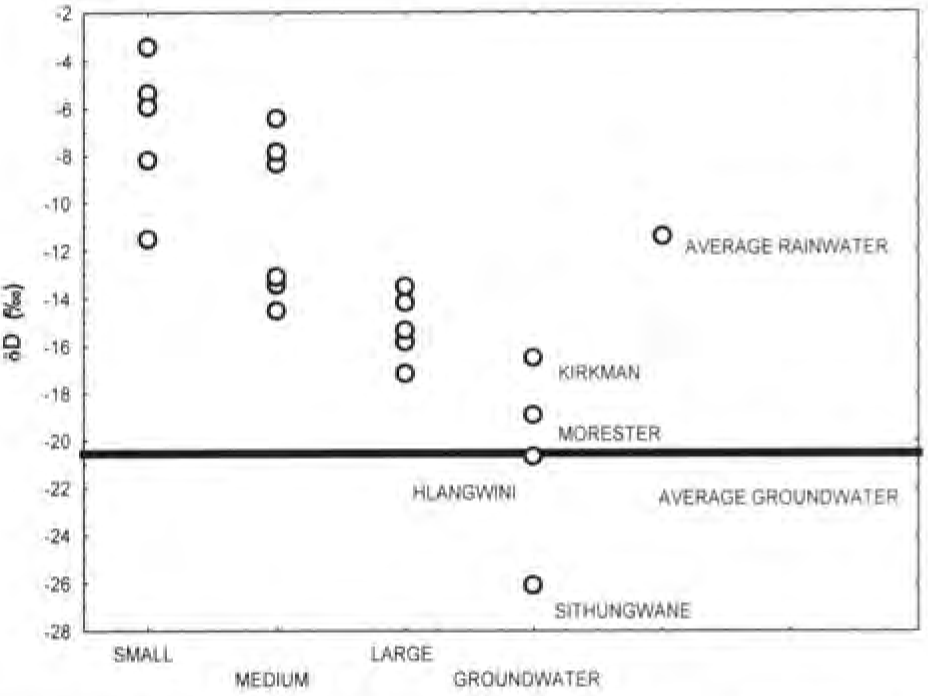

Figure 6: Hydrogen isotope ratios for small, medium, and large individuals of *T. sericea*. The average isotope value of each borehole, namely Kirkman, Morester, Hlangwini and Sithungwane, over April, May and June are plotted. The solid line represents average isotope ratio of groundwater over the 3 month period. The average isotope value of rainwater April, May and June are also plotted.

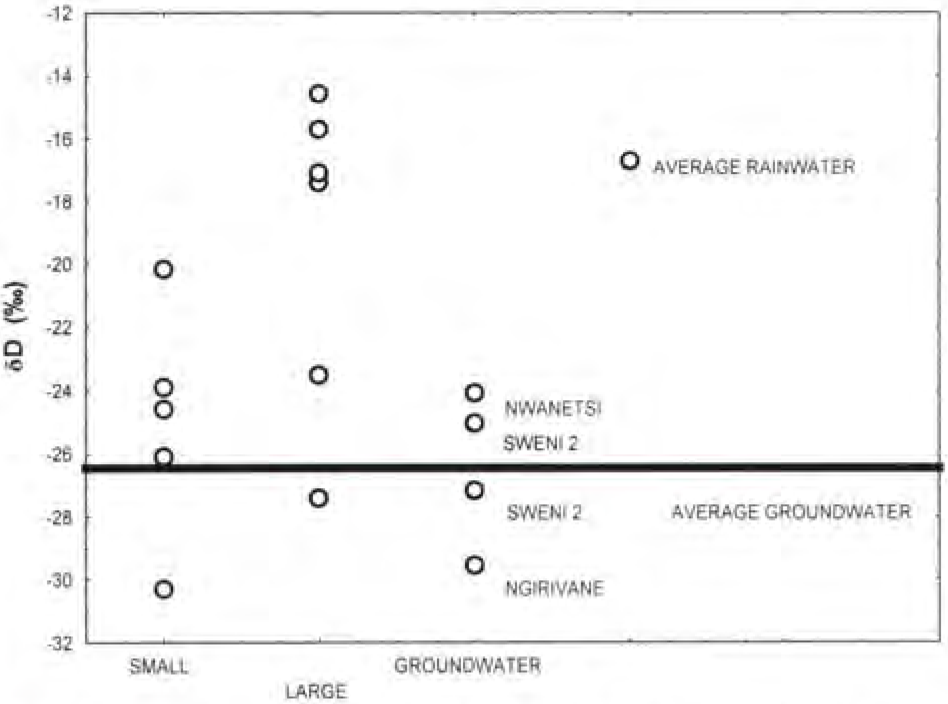

Figure 7: Hydrogen isotope ratios (δb) for small and large individuals of *A nigrescens* at Satara. The average isotope values of each borehole, namely Nwanetsi, Sweni 1, Sweni 2, Ngirivane, over April, May and June are plotted. The solid line represents average isotope ratio of groundwater over April, May and June. The average isotope value of rainwater for the three months is also plotted.

DISCUSSION

Typically an isotopic gradient occurs through the soil profile, with soil water in the surface layers tending to be more isotopically positive and water in deeper layers tending to be more negative in isotopic composition (Allison *et al.*, 1983). Two main factors cause this isotopic gradient to occur through the soil profile. Groundwater is usually recharged by the local precipitation and there may be selective recharge either during heavy rain (Mazor & Verhagen, 1983) events or selective recharge of certain forms of precipitation (e.g. hail) (Dawson, 1996). Rainfall is usually more negative in its isotopic composition during heavy rainfall events because there is less opportunity for rainfall to evaporate (Dansgaard, 1964). Different precipitation forms usually have different isotopic compositions. For example hail is typically more isotopically negative than rainfall, which is primarily due to the temperature at which the precipitation is formed. The second factor that can cause water deeper in the soil profile to be isotopically negative than shallow water is evaporation. Evaporation of water is greater in the surface layers, and during evaporation there is preferential loss of the lighter isotope. Thus evaporation of water from soil typically causes surface layers to be more isotopically positive than deeper soil water or groundwater (Allison *et al.*, 1983). We tried to test this by taking soil samples from different depths (table 2) and attempting to analyze the isotopic composition of soil at various depths. However the current water extraction line is setup for extracting xylem water from twig samples, and the Kimax® tubes were unable to hold enough soil to extract sufficient water (2 ml) to analyse the sample water's hydrogen and oxygen isotopes. Furthermore the test tube-like shape of Kimax® tubes is not optimal for extracting water from soil, because water evaporates from the sample in Brownian motion and thus because the tube is full of soil and it has a very small surface area from which to evaporate, water cannot evaporate easily from the soil. Thus a modification would need to be made to the line, particularly for soil samples with a low water content, which would allow a greater surface area of the soil to be exposed. A better shape would probably be a bulb, but this would mean running fewer samples (four instead of six) at a time.

The isotopic composition of groundwater from each borehole was relatively stable over the three month period of this study (table 3). This is because groundwater is an accumulation of various rainfall events and thus represents a large body of water. Evaporation is unlikely to change the isotopic composition of groundwater much because the water table is deep in the soil profile (table 1), deeper then 8 meters at

Pretoriuskop and more than 4.5 meters deep at Satara. Furthermore with April, May and June being winter months the amount of rainfall would have been low and thus it is likely that little or no rainwater was contributing to groundwater during these months.

Although the isotopic composition of groundwater was relatively stable for each borehole over the study period, the isotopic composition did vary slightly between boreholes at each site (table 3). For example at Pretoriuskop, Kirkman borehole, the closest to the tree sampling site was the most isotopically positive (δD = -16.5 ± 1.0 ‰) and there was a progressive decrease in both δ^{18}O and δD values to furthermost borehole, Sithungwane (δD = -26.1 ± 0.9‰). And at Satara the three boreholes close to one another, namely Sweni 1 (δD = -27.2 ± 6.2‰), Sweni 2 (δD = -25.0 ± 1.1‰) and Nwanetsi (δD = -24.1 ± 1.4‰) had similar isotopic compositions that were different to the far away Ngirivane borehole (δD = -29.6 ± 0.9‰). This variation in the isotopic composition of the boreholes at sites cannot be explained by the input of precipitation with different isotopic composition or by different soil types because they are only a few kilometers apart. However the difference between boreholes can be explained by different infiltration rates at the sites (a slow infiltration rate offers more opportunity for evaporation and thus alteration of the isotopic composition). Underlying geological features can alter the drainage of the soil resulting in different evaporation rates from soil.

The variation in isotopic composition of boreholes was greater when comparing boreholes between sites than within sites (Figure 5). Pretoriuskop boreholes (δD = -20.5 ± 3.8‰) had significantly more positive hydrogen isotope values than either Satara boreholes (δD = -26.4 ± 3.3‰) or Shingwedzi boreholes (δD = -29.3 ± 3.4‰). And although there was no significant difference in the isotopic composition of boreholes at Shingwedzi and Satara boreholes, boreholes at Shingwedzi did have a slightly more negative isotopic composition compared to Satara boreholes. This can be explained by different infiltration and evaporation rates from different types of soils (for example clays endure less evaporation than sandy soils, because water is attracted to clay particles. Thus when rain falls and infiltrates into the soil in sandy soils such as at Pretoriuskop evaporation will typically be higher than the evaporation from water infiltrating through clay soils, such as at Satara. Thus the more isotopically positive hydrogen and oxygen ratios at Pretoriuskop compared to Satara could be explained by the presence of sandy soils at Pretoriuskop and clayey soils at Satara. Furthermore isotopic differences between groundwater at the study sites could also be explained by a difference in the isotopic composition of precipitation.

The isotopic composition of precipitation can be altered by four main factors, namely temperature, altitude, amount, and continentality (Dansgaard, 1964). The temperature can affect the isotopic composition of precipitation because at high temperatures the lighter isotope preferentially evaporates enriching precipitation with heavy isotopes. Thus the warmest months should produce rainfall with the highest oxygen and isotope isotopes ratios (Dansgaard, 1964). However this effect is unlikely to be observed at KNP because the temperature does not change much over the year, and also the effect is likely to be masked by the amount effect. The amount effect can change the isotopic composition of rainfall because when larger amounts of rain fall, oxygen and hydrogen isotope ratios are typically more negative (Dansgaard, 1964). This is because during periods of heavy rainfall there is usually less opportunity for evaporation of rainwater prior to reaching the ground. Winter months are typically the dry months in KNP and thus any precipitation occurring during the study period from April to June would likely have been light precipitation events. Thus the amount effect can explain why rainfall isotopic composition was more positive relative to the isotopic composition of groundwater (Figure 5). The altitude effect is caused by cooler temperatures at higher altitudes and therefore δD and $\delta^{18}O$ values of precipitation should be more negative at higher altitudes. However the topography of KNP is quite flat and altitudes only range from 600 m at Pretoriuskop to about 275 m at Satara and 350 m at Shingwedzi. Thus altitude effect is unlikely to play a major role in influencing the isotopic composition in KNP. As precipitation moves over-land δD and $\delta^{18}O$ values of each precipitation event usually becomes move negative (Dansgaard, 1964). This is known as the continentality effect and is caused by heavier isotopes preferentially precipitating out under the influence of gravity, leaving the water left behind in the cloud depleted of heavy isotopes and thus more negative in terms of δD and $\delta^{18}O$ values. The combination these four factors usually causes variation amongst the hydrogen and oxygen isotope ratios of precipitation and thus groundwater, because precipitation recharges groundwater.

At the Pretoriuskop site the hydrogen isotope ratios from large *T. sericea* individuals (δD = -15.2 ± 1.4‰) was similar to hydrogen isotope ratios from the closest borehole, Kirkman (δD = -16.5 ± 1.0‰). This suggests that large trees were able to develop a deep root structure able to access groundwater and were utilizing this water. Medium-sized individuals (δD = -10.6 ± 3.5‰) had a similar isotopic composition to that of average rainfall (δD = -11.4 ± 4.9‰). This suggests that medium-sized individuals were utilizing rainwater or soil water. Some medium-sized individuals may have had some access to groundwater but most individuals showed a greater reliance on rainfall (observable by their isotopic shift towards rainfall) than large trees. Small *T. sericea* individuals (δD = -6.9 ± 3.1‰) showed a more positive isotopic composition than average rainwater (δD = -11.4 ± 4.9‰) and thus must have been utilizing an evaporated water source. It is likely that small individuals were utilizing rainwater or very shallow soil water that had undergone extensive evaporation.

At the Satara site most large *A. nigrescens* individuals (δD = -19.3 ± 5.0‰) had a similar isotopic compositions to that of average precipitation (δD = -26.4 ± 3.4‰). This suggests that large *A. nigrescens* trees have a shallow root system that is only able to utilize rainwater and shallow soil water. This notion of a shallow root is supported by observations that elephants manage to push over adult *A. nigrescens* trees with apparent ease (van Wyk, 1974; Rademan, pers. comm.). Unexpectedly *A. nigrescens* gullivers (δD = -25.0 ± 3.7‰) had a similar isotopic ratios to that of average groundwater (δD = -26.4 ± 3.4‰). This suggests that *A. nigrescens* gullivers had access to groundwater. This is supported by a recent excavation of a gulliver which found a deep tap root (Rademan, pers. comm.) excavated a gulliver and found tap root that went down. However when adult individuals are pushed over by elephants they do not appear to have this tap root (Rademan, Pers. comm.). One explanation may be that gullivers are sensitive to drought and thus they develop a tap root so that they can access a permanent source of water whilst they are establishing. However when the trees are establish beyond the fire trap they optimize water uptake and this is done by having a shallow root system that is able to quickly take up rainfall. Not only does this allow the tree to outcompete other deep rooted tree species where the water table is deep, the tree can take up the water before it becomes incorporated into the soil structure, such as with clay soils. Adults are not usually found near a water source (van Wyk, 1974) and this may be because they have a shallow root and don't use groundwater and therefore may be outcompeted by other species using this water source. Another explanation for the apparent access of gullivers to groundwater is the observation that gullivers often occur on deeper soils whereas adults often occur on shallower soils (Rademan, pers. comm.). Thus gullivers are able to develop a root structure that is able to deeply penetrate the soil structure where there is little evaporation of water. Adults occur on the shallower soils that are likely to be better drained during the rainy season, whereas the deeper soils that gullivers usually grow on may become seasonally waterlogged and this would limit their development.

Conclusion

Many studies have looked at the partitioning of water between grasses and trees, whilst comparatively few have investigated whether different trees and gullivers are sourcing the same water. If gullivers and trees are sourcing different water this will have to be incorporated into models attempting to model the establishment stage of savanna trees.

Isotopic evidence from boreholes suggests that *T. sericea* trees are utilizing groundwater, and thus have the ability to develop a deep root structure that is able to access groundwater. Furthermore medium-sized individuals may be using some groundwater and some rainwater and small gullivers utilizing rainwater or soilwater that has undergone extensive evaporation. Thus isotopic data from this species supports the hypothesis that large *T. sericea* individuals have access to groundwater whilst the gullivers are restricted to utilizing rainwater. Thus with *T. sericea* fire as well as access of gullivers to rainwater is important in determining the establishment of this species. Isotopic data from large *A. nigrescens* is shallow rooted and are mainly sourcing rainwater and not groundwater. However isotopic evidence from gullivers suggests that gullivers have some unexpected access to groundwater. Thus the ability of savannas trees and gullivers to access groundwater is a species-specific phenomenon that depends on the ability of the gulliver or adult to develop a deep root structure. This study shows that adults and gullivers are sourcing different water and this needs to be taken into account when attempting to model savannas.

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APPENDIX 1

Table A: Size parameters of small, medium and large *T. sericea* trees at the Pretoriuskop study site. Stem diameter was recorded as diameter at breast height for large and medium individuals and the diameter at the base of the stem for small individuals.

Size category	Tree code	Living ht (m)	Top kill Ht (m)	Max canopy diameter (m)	Perp. canopy diameter (m)	No. of stems	Total stem diameter (mm)
Large	TS35	10		5	5	1	290
Large	TS86	10		5	3.75	1	270
Large	TS43	6.5		6.5	6.5	1	280
Large	TS77	7.5		5	3.5	1	290
Large	TS39	8		7.5	7.5	1	420
Large	TS85	8		12.5	10	1	420
Medium	TS005	2.5	2.8	1.1	0.9	1	63
Medium	TS006	2.75	3	0.7	0.5	1	50
Medium	TS80	3.5	3.8	2	1.8	2	113
Medium	TS93	3.5	3.8	2.5	2	2	78
Medium	TS94	3		2.5	2	4	176
Medium	TS008	4		1.7	1.4	1	60
Small	TS92	0.6	1.5	0.7	0.4	3	17
Small	TS91	1.1	2.25	0.7	0.65	3	32
Small	TS97	0.9	1.9	1.3	1.2	5	43
Small	TS98	0.7	1.5	1.1	0.9	5	45
Small	TS95	0.7	2.1	1.1	0.8	6	33
Small	TS44	0.9	1.3	1.3	0.9	10	59

APPENDIX 1

Table B: Size parameters of small and large *A. nigrescens* trees at the Satara study site. Stem diameter was recorded as diameter at the base of the stem for both small and large individuals.

Size category	Tree code	Living ht (m)	Top kill Ht (m)	Max canopy diameter (m)	Perp. canopy diameter (m)	No. of stems	Total stem diameter (mm)
Large	AN78	3		1.5	1.5	1	255
Large	AN140	4.5		1.7	1.5	1	320
Large	An139	5		1.2	1	1	170
Large	AN133	5		1.3	1	1	240
Large	AN153	4		3.5	3	1	
Large	AN91	6.25		3.5	3.5	1	190
Small	AN131	0.75	1.8	1.6	1.5	5	48
Small	AN77	0.8	1.4	1.6	1.4	9	114
Small	AN73	0.75	1.75	1.3	1.3	3	41
Small	AN134	0.7	1.4	1.4	1.7	5	67
Small	AN135	0.7	1.8	1.5	1.8	11	110
Small	AN79	1	2	2	1.8	11	142