Do different size classes of trees access different water sources?
An example from an African Savanna

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1. Abstract

Walter's (1971) root niche hypothesis predicts the coexistence of trees and grasses by the utilisation of superficial soil layer by grasses and deeper soil layers by trees. The model ignores the fact that tree seedlings are competing for the same water source as grasses during the establishment phase. This may also hold true for juvenile trees that are still in the process of laying down root reserves to access deep groundwater. This means that juvenile trees may be reliant on a different water source to adult trees and could potentially also be more water stressed as their competing directly with grasses for the same surface soil water. There may also be no space for root niche separation in shallow soils, forcing trees of all sizes to share the same water source with grasses.

I tested whether different sized trees access the same water source as predicted by the root niche separation model proposed by Walter (1971). δD and δ^{18}O values indicate that small, medium and large size classes of *A. nigrescens* access the same water source. For *T. sericea,* δD and δ^{18}O values suggest that large trees have access to an alternative deeper water source from that utilised by medium- and small-sized trees. Xylem pressure potential suggests that large individuals of both species are significantly more water stressed during the dry season compared to small and medium sizes.

The Walter hypothesis (1971), considers that trees and grasses exploit different layers in the soil horizon. He further predicts that that soil type will predict tree density on the basis of variable water availability at different depths in the soil. The present study on two different soil types, suggests that trees compete with grasses in the juvenile life stages. Large trees at Pretoriuskop (well drained sandy soils) access a different water source from the juveniles; the same class at Satara (poorly drained clay soils) uses the same water as juveniles. As a consequence root niche separation cannot predict tree densities across both sites, thus it cannot explain the phenomenon of tree grass coexistence of savannas.

2. Introduction

Savannas are ecosystems defined by the coexistence of two contrasting life forms, trees and grasses such that there is a continuous grass and a discontinuous layer of trees within these systems (Knoop and Walker, 1985). A central question in savanna ecology is how these two contrasting life forms co-exist without one dominating over the other (Scholes and Archer, 1997; Knoop and Walker, 1985; Walter 1971). Currently there are two contrasting hypotheses that explain the relationship between grasses and trees in savannas. The first, initially developed by Walter (1971) and then later by Walker and Noy-Meir (1982) emphasizes bottom-up controls such as water. The second by Higgins *et al.* (2000) considers top-down controls such as fire.

The classic competition-based model proposed by Walter (1971), considers that trees and grasses exploit different layers within the soil horizon. This enables them to coexist as grasses utilize surface water whereas larger trees utilises deeper ground water. Walter (1971) further predicted that soil type would be a predictor of tree density, based on the availability of water at different depths in the soil. He also predicted that soils that are well drained should support a greater number of trees as the deeper water will only be available to trees. At the same time there will be less water available in the upper layers to support grasses.

Walker and Noy-Meir (1982) further developed this root niche separation model, which assumes that a given set of environmental, abiotic conditions will result in a predictable proportion of trees and grasses.

In contrast the demographic-bottleneck hypothesis first proposed by Higgins *et al.* (2000) argues for a mechanism whereby trees and grasses coexist due to top-down controls such as fire and herbivory (Sankaran *et al.*, 2004; Higgins *et al.*, 2000). In this model, seedling establishment are inhibited by seasons of insufficient rainfall and competition with grasses (Polley *et al.* 1997). If establishment is successful, juvenile plants are consistently 'knocked back' by frequent grass fires; this prevents recruitment in to the next size class. Grasses and trees thus coexist as trees are prevented from reaching maturity by frequent fires that continuously remove their prevented from reaching maturity by frequent fires that continuously remove their aboveground biomass. The trees are thus trapped as gullivers in the fire zone, resprouting again and again, but never reaching maturity unless a long enough fire-free period occurs. Stochastic events such as above average rainfall can result in dominance of trees over grasses (Archer, 1989), but there is no equilibrium point between these two end member outcomes.

Thus for trees to persist, rainfall and fire severity through time needs to be sufficiently variable to allow periodic years suitable for recruitment (Higgins *et al* 2000). In addition adult trees must survive and continue to set seed between periods suitable for recruitment. If both of these criteria are met, then a dynamic balance between trees and grasses can be maintained. If fire frequencies/severities are reduced, recruitment for trees would increase and grasses will shaded out. If there are too may drought years and high intensity fires, then trees will be unable to recruit and the system will tend toward grassland.

Recently, the demographic type hypotheses have gained favour over the root niche hypothesis (Sankaran *et al.,* 2004). There are two main reasons for this: firstly, attempts to model co-existence in spatially explicit models based on field data, using resource competition alone as a mechanism, have been unsuccessful (Jeltsch *et al.* 2000). Secondly, empirical support for root niche separation in water uptake by trees and grasses has for the most part been equivocal (Sankaran, 2004; Walker and Noy-Meir, 1982).

In this experiment I closely examine the assumption made by the root separation model (Walter, 1971) that trees and grasses use different water sources, by looking at different size classes of trees. The model ignores the fact that tree seedlings are competing for the same water source as grasses during the establishment phase. This may also hold true for juvenile trees that are still in the process of laying down root reserves to access deep groundwater. This means that juvenile trees may be reliant on a different water source to adult trees and could potentially also be more water stressed as their competing directly with grasses for the same surface soil water. There may also be no space for root niche separation in shallow soils, forcing trees of all sizes to share the same water source with grasses.

It has been established that when plants take up water, there is no fractionation of the isotope from the source water (Dawson & Ehleringer, 1991) That means that $\delta^{18}O$ and δD signature of the source water and the water in the plant are identical. Zimmerman *et al.* (1967) was probably the first to show that evaporation of the surface layers of a saturated soil column causes an enrichment of δD and $\delta^{18}O$ that decreases as one moves down the column. If trees are using different layers of the soil horizon for water than grasses, then the δD and $\delta^{18}O$ values of the xylem water of small, medium and large trees should all reflect the same δD and $\delta^{18}O$ values. This is because according to the root niche hypothesis, these trees will all be sourcing a deep water source which will have a different isotopic value to the surface water.

For this study water sourcing will be assessed by direct comparison of twig xylem water δD and $\delta^{18}O$ values, leaf $\delta^{13}C$ and xylem pressure potentials for three different size classes of two tree species, *A. nigrescens* (occurring on shallow soils) and *T. sericea* (occurring on deep soils). Samples from three potential water sources (rainfall, boreholes (analogue to ground water table) and soil water) were collected, to help in assessing where in the soil layer, the different size classes are sourcing their water from.

3. Methods

3.1. Site Description

The Kruger National Park (KNP) is situated in the Lowveld of southern Africa, in the east of South Africa's Northern and Mpumalanga Province. Established in 1926, the park comprises 20 000 km^2 and is the largest game reserve in South Africa. The KNP is cut roughly in two along a north-south line by geology, such that broadly speaking, basaltic soils are found in the east (Satara) and granitic soils in the west (Pretoriuskop). The climate is subtropical, with maximum daily temperature ranging between 26°C and 33°C. Most of the rain falls between September and March and there is very little precipitation between May and August. Two study sites were selected; Satara: with shallow, basaltic derived soils and Pretoriuskop, with deep, granite derived soils.

3.1.1. Pretoriuskop

The Pretoriuskop area is characterised by the presence of granite outcroppings and has a mean annual rainfall of 719 mm (du Toit *et al.*, 2003). *Terminalia sericea* is the dominant tree here, which has an exclusive preference for deep well-drained soils and its presence is closely related to sandy soils (Palgraves, 2002). The soils at the Pretoriuskop site are deep (>1m), nutrient poor, sandy and deep red in colour. The sampling was carried out in an exclosure north west of the Pretoriuskop rest camp.

3.1.2. Satara

Acacia nigrescens, is the dominant tree species at this site, but grows on all geological fromations found within the KNP (van Wyk 1974). Sampling was carried out in an exclosure neighbouring the Satara buffalo exclosure. Soils here are shallow (<0.75m), comprised of nutrient rich clay, with a dark brown colour. The mean annual rainfall is 540 mm (du Toit *et al.*, 2003).

3.2. *Size Classes*

In May 2003, two distinct size classes were identified for *A. nigrescens* at Satara and three for *T. sericea* at Pretoriuskop. The size classes were determined by canopy height and six individuals in each size class tagged. Measurements of size were recorded for each tagged individual. I repeated the 2003 size measurements done by Robert Taylor in April and August 2004 for the tagged individuals of both *A. nigrescens* and *T. sericea.* I also added an additional size class (large) for *A. nigrescens* in August 2004. Stem diameter was determined using a diameter tape at the base of the small-size class trees and at breast height for medium- and large-size class individuals. The different size classes were compared by canopy height and summed basal diameter to verify whether the size classes were meaningful and comparable between species.

3.3. *$\delta^{18}O$ and δD isotopic analysis*

3.3.1. Water and Hydrogen Extraction

Oxygen was extracted from rain, borehole, soil and stem water, using the CO_2 equilibrium method (Socki et al. 1992). Water (1.5 ml) from each of the samples was injected into 7 ml Vacutainers® prefilled with CO_2 to 500mbar. The Vacutainers® were then placed in a rocking apparatus, in a water bath at 25°C ca. 24h. This facilitates an equilibrium exchange process, allowing the oxygen isotope signal of the sample to be reflected in the oxygen of the carbon dioxide gas.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O \qquad (1)$$

The CO_2 was then extracted from the water sample using a cryogenic distillation process (Craig, 1961) process involving a system of U-tubes. Alternating emersion of the sample in liquid Nitrogen and a liquid Nitrogen/ethanol slush were used to eliminate non-condensable gases, such as oxygen and nitrogen from the sample.

The zinc-reduction method outlined by Coleman et al. (1982) was used to separate H_2 from the water sample. This involves introducing 2µl of water sample in a capillary tube into a glass tube filled with ca. 100 mg of zinc shavings. The tube is then attached to a vacuum line, frozen with liquid N_2, and then evacuated to eliminate non condensable gases such as nitrogen and oxygen. The glass tubes are then sealed with and oxy-acetylene torch. Prior to analysis on the mass spectrometer (Finnegan MAT252), the samples were placed in a furnace @ 500°C for 1h to reduce the water to Hydrogen and Zinc Oxide, according the equation:

$$Zn + H_2O \rightarrow ZnO + H_2 \qquad (2)$$

The values are expressed as δD and $\delta^{18}O$ (‰), relative to the accepted marine Ocean Water Standard (V-SMOW), via the equations of Coplen (1993). Ocean water is enriched relative to most natural terrestrial compounds, most substances have negative δD and $\delta^{18}O$ values. $^{2}H/H$ ratios are calculated relative to the ocean waters standard from the equation:

$$\delta D = ((^{2}H/H\text{sample})/(^{2}H/H\text{std}) - 1) * 1000‰$$

3.3.2. Water Isotopes: Meteoric Water Line

The δD and $\delta^{18}O$ data for all meteoric water samples (rain and borehole) from both Pretoriuskop and Satara was plotted, and fitted with a trend line designated as the local meteoric water line (LMWL). The global meteoric water line (GMWL) ($y = 8x + 10$) is also depicted. Isotopic values for soil water from Pretoriuskop and Satara was also plotted on the same graph, but not included in the regression.

3.3.3. Borehole

Four decommissioned boreholes around the Pretoriuskop enclosure (Kirkman, Morester, Hlangwini, Sithungwane) and four boreholes around the Satara enclosure

(Nwanetsi, Sweni1, Sweni2, Ngirivane) have been sampled monthly from February 2003 to July 2004. Water was collected in Vacutainers ® (7ml airtight glass vials) and sealed with Parafilm ® to prevent fractionation of the samples.

3.3.4. Rainfall

Rainfall is collected at monthly intervals from the permanent rain water collectors at the weather stations of both the Satara and Pretoriuskop enclosures. The monthly samples are stored in Vacutainers ® and sealed with Parafilm ®. Rain water collectors were constructed using 500ml jars covered with mesh. Paraffin and silica gel is placed in the jars. Since paraffin and silica gel have a lower density than water, the rainwater that is collected is covered by a layer of silica or paraffin which prevents evaporation. This prevents the fractionation of the rain water collected.

3.3.5. Soil

Soil samples were collected from two similar locations at each of the Pretoriuskop and Satara enclosures. The Satara soils samples went down to 0.75cm and were sampled at intervals of 15-25 cm, 50-60cm and 65-75cm. The Pretoriuskop site was sampled at 10-20, 30-40, 50-60 and 80-90cm intervals along the auger profile. Each soil sample was placed in two Ziploc plastic bags and stored in a cooler to prevent moisture loss. Only once the samples were back in the Cape Town laboratory were the samples frozen down to prevent freeze-thaw fractionation. Prior to water extraction a small (<50g) sample of soil was dried at 70 °C for 48 hours to determine gravimetric soil water content (%). This was used to determine the mass of soil required to extract 2m*l* of water for CO^2 equilibrium method (Socki *et al.*, 1992).

3.3.6. Water Isotopes: Sapwood

I analysed the $\delta^{18}O$ and δD composition of water extracted from stems of individuals of *A. nigrescens* and *T. sericea* from the different size classes for May 2003, April and August 2004. Two one-centimetre diameter twigs with a length of 5 centimetres were cut from each individual of *T. sericea* and *A. nigrescens* in all size classes. These twigs were sealed in a Kimax ® tube and the lid sealed with Parafilm®. This was done to prevent fractionation of the water in the twig with water in the atmosphere between collection and the laboratory. The Kimax ® tubes fit directly into the extraction line which minimizes potential sources of contamination.

For this analysis, I wanted to compare how the isotopic water values for the stems related to different water sources (rain versus groundwater). To do this, I compared the isotopic water values for the stems to an average isotopic borehole value (analogue to groundwater) from Satara and Pretoriuskop for the month immediately prior to the collection of sapwood samples. This was not done for rainfall as there was often no isotopic value for the month in question and where data was available the small sample size (n=1) made it difficult to rely on the value.

3.4. Stable Carbon Isotopes

Leaf $\delta^{13}C$ can be used as an analogue for plant water use efficiency (WUE). (Farquhar, 1982). Farqhar *et al* (1988) have formulated the theoretical base for plant fractionations in the following equation.

$$\delta^{13}Cp = \delta^{13}Cair - a - (b-a) \times c_i/c_a$$

Where: $\delta^{13}Cp = \delta^{13}C$ in the plant

$\delta^{13}Cair = \delta^{13}C$ of the air

a = Stomatal diffusion factor (4.4‰)

b = Rubisco fraction factor (27‰)

$c_i = CO_2$ conc. of air inside leaf

$c_a = CO_2$ conc. of atmosphere (-8‰)

The ratio of ^{13}C to ^{13}C in a sample is composed relative to an internationally accepted carbonate standard derived from belemnite fossils of the Pee Dee formation (Craig, 1957). Carbon $^{13}C/^{12}C$ is calculated relative to this limestone from the equation;

$$\delta\, ^{13}C = \ (^{13}C/^{12}Csample)/(^{13}C/^{12}Cstd) - 1) * 1000‰$$

Relative to the PDB the $^{13}C/^{12}C$ ratio of the sample is designated as and given as $\delta\, ^{13}C‰$ (Craig, 1957).

The only factor that is not a constant is the CO_2 concentration in the leaf, and that is a function of how widely the stomata are opened. Such that more negative $\delta^{13}C$ values are indicative of lower water stress.

T. sericea and *A. nigrescens* are both deciduous, dropping their leaves at the end of each dry season. New leaves are put on at the beginning of each wet season, using stored carbohydrate from the previous growth season. So the $\delta^{13}C$ values of the new leaves will give an idea of the relative stress experienced by different size classes in the previous growing season.

The youngest looking leaves were sampled from each tree. I stored the leaves in separate small, Ziploc containers with silica gel to desiccate the samples. The leaves were ground in a hammer mill and 60 to 80µg of each sample was weighed out and combusted in an elemental analyser (Model NA 1500; Carlo Erba, Milan Italy).

3.5. Pressure Bomb

A PMS instruments pressure chamber was used to measure xylem pressure potentials. Scholander *et al.* (1965) demonstrated that a pressure chamber could be used to evaluate the status of water within vascular plants. The technique measures the pressure required to force water back to the cut surface of a severed twig (Waring *et al.* 1967).When a twig is cut, the water column that is under stress withdraws a short distance up the stem. When the leafy end of the twig is placed in the pressure chamber, the pressure at which water is seen at the cut is assumed to be the reciprocal of the tension that the water column was under before it was cut.

Xylem Pressure Potential (XPP) was measured twice daily, predawn (3- 5 am) and midday (11 am – 3pm) for all tagged individuals of both species in April and August 2004 (except in April 2004 where only predawn was measured for *T. sericea*). The predawn measurements give an indication of the base line tension that the plant is returning to each day. Midday measurements indicate the maximum stress that the plant is under during the peak temperature of the day. The difference between the midday and predawn pressure potentials decreases as drought stress increases (Waring, 1967).

3.6. Statistical analysis

A one way ANOVA was used to compare mean ouput for the different size classes. Where the assumption of homogeneity of variances (tested by the Levene and Brown-Forsythe test) was violated a Mann-Whitney test was perfromed. To test which classes were significantly different from each other, a Tukey post-hoc test was applied.

4. Results

4.1. Size Classes

Repeat size estimations for the different classes made in April and August 2004 were not consistent with the size measurements made in 2003 or with each other. This is probably due to differences in measuring protocol between recorders. In addition small size classes had stems removed for stable water isotope analysis in May 2003 and April 2004 which affected the size measurements. Therefore, to compare between size classes, the original May 2003 size groupings were used.

Fig. 1a show that there are three distinct size classes for both *A. nigrescens* and *T. sericea* and that these size classes are similar between species although not always significantly so. Canopy height (m) (Fig. 1a) and summed basal area (mm^2) (Fig. 1b) was found to be significantly different between all size classes for both *T. sericea* (canopy height: p = 0.000, basal area: p = 0.000) and *A. nigrescens* (canopy height: p = 0.000; basal area: p = 0.014). Canopy height was similar for the small size-class of *A. nigrescens* and *T. sericea* (p = 0.7124) trees (Fig. 1a), but summed basal area was significantly different (p = 0.004) (Fig. 1b, not clearly seen due to compression of the y-axis by the variation in the larger size classes).

Comparisons between the medium size-classes of *T. sericea* and *A. nigrescens* showed that both canopy height (p = 0.0179) and summed basal area (p = 0.004) were significantly different. For the large size-class there was a significant difference between canopy heights (p = 0.0193) but a non-significant difference between summed basal area (p = 0.81).

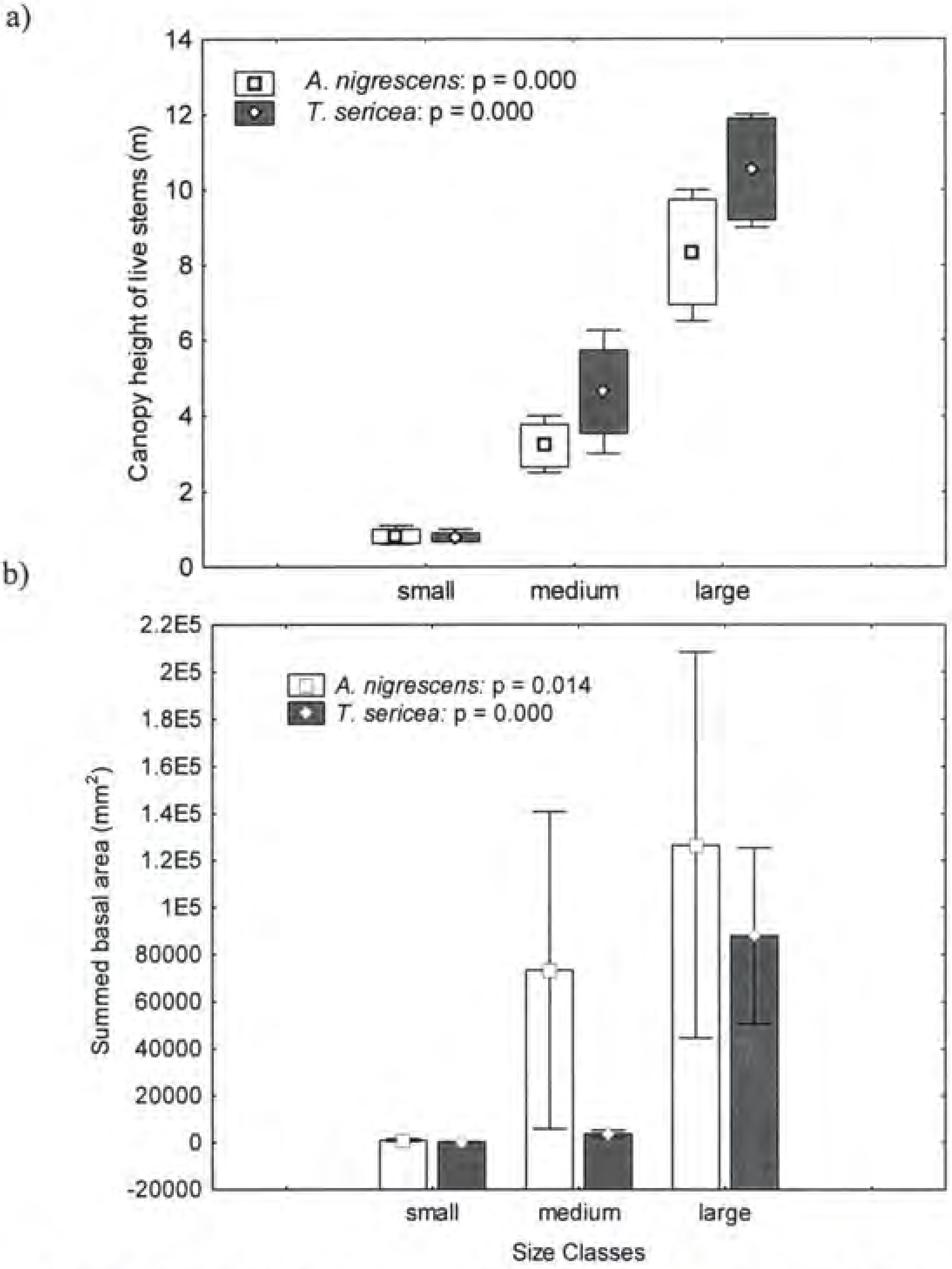

Figure 1 Canopy height of live stems (m) (a) and mean summed basal area (mm^2) (b) for the three defined size classes of *A. nigrescens* at Satara and *T. sericea* at Pretoriuskop. For mean summed basal area (mm^2) and canopy height, the size classes were significantly different within species for both *T. sericea* (basal area: p = 0.000; canopy height: p = 0.000) and *A. nigrescens* (basal area: p = 0.014; canopy height: p = 0.000). Mean summed basal area (mm^2) was significantly different between the small (p = 0004) and medium (= 0.004) size classes of *A. nigrescens* and *T. sericea* but not for the large size class (p = 0.81). Canopy height was significantly different between medium (p = 0.0179) and large (p = 0.0193) size classes for *A. nigrescens* and *T. sericea* but not for the small size class (p = 0.7124).

4.2 Meteoric Water Line

There was a significant relationship between $\delta^{18}O$ and δD values of rain, borehole and soil water for both Pretoriuskop ($R^2 = 0.38$, $p = 0.00004$) and Satara ($R^2 = 0.39$, $p = 0.00001$).

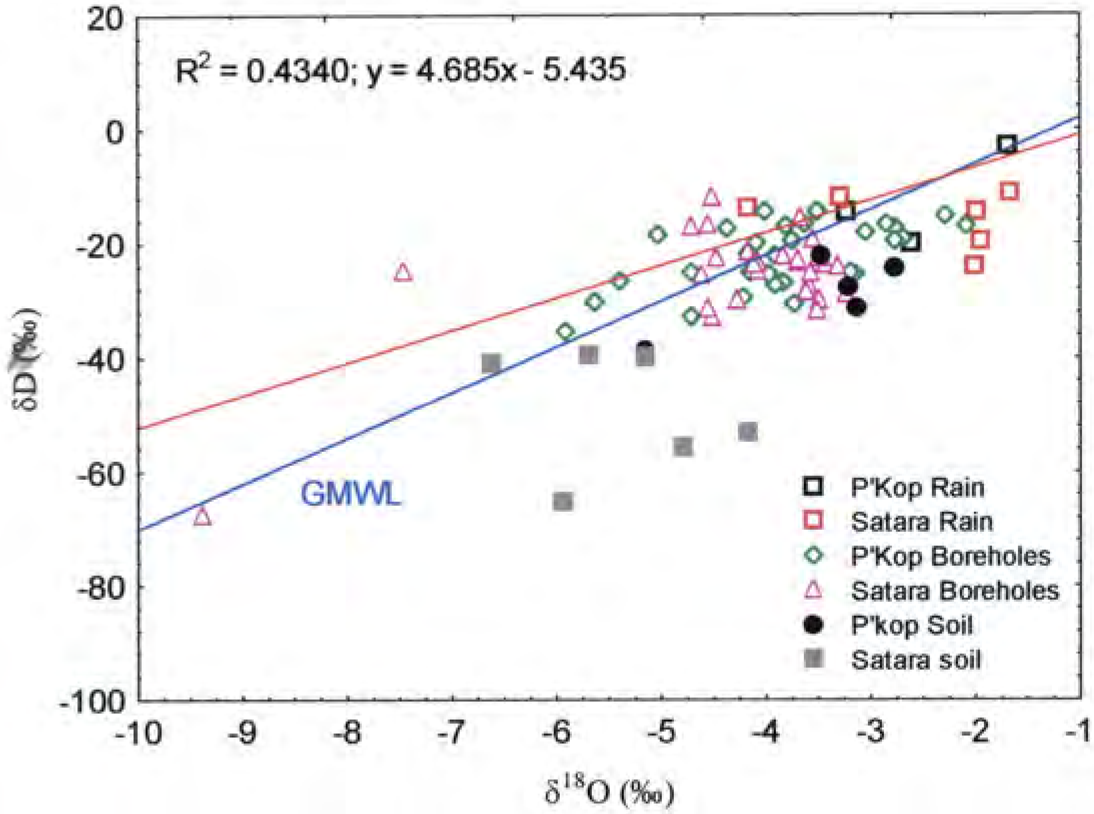

Figure 2: Meteoric water line (red) ($R^2 = 0.434$, $p = 0.000$) for Satara and Pretoriuskop using rain and borehole data from February 2003 to July 2004. Soil values for April 2004 only. Soil values are not included in the regression. The Global Meteoric Water Line (GMWL), in blue is added as a reference.

When the rainfall, soil water and borehole water $\delta^{18}O$ and δD values for Pretoriuskop and Satara were combined, the rainfall values were similar between the two sites (Fig. 2). The range of isotopic values overlapped, barring a few outliers, for both the Pretoriuskop and Satara boreholes (Fig. 2). There was a general trend for the rainwater values to have more positive values than the boreholes for Pretoriuskop, whereas the rain and borehole water values for Satara were more closely grouped (Fig. 2). The Pretoriuskop soil isotope values were nested between the Pretoriuskop rain and borehole values, indicating that rainfall may be fractionated as it moves through the soil profile towards the groundwater table (Fig. 2). In contrast, the soil water isotopic values at Satara separated out distinctly from both the borehole and rainfall values, suggesting that there is strong fractionation occurring within the soil (Fig. 2).

4.3. Water Isotopes: sapwood

4.3.1. *Acacia nigrescens*: between size classes

For *A. nigrescens* there was no difference between the $\delta^{18}0$ values of any of the size classes in the April ($p= 0.756$) or August ($p = 0.630$) 2004 sampling (Fig. 3a). The May 2003 ($p = 0.043$) sampling showed a significant difference between the small and medium size classes ($p = 0.009$).

The δD values for *A. nigrescens* showed no difference between any of the size classes for May 2003 ($p = 0.1989$), April ($p = 0.8926$) or August ($p = 0.5607$) 2004 (Fig. 3b). These results suggest that apart from the $\delta^{18}O$ difference between small and medium size classes in May, *A. nigrescens* individuals of all size classes are accessing the same water source.

4.3.2. *Acacia nigrescens*: borehole

The mean Satara borehole $\delta^{18}O$ value for May 2003 was -3.85‰, for April, -5.94‰ and August -3.63‰ (Fig. 3a). The δD value for May 2003 was -26.93‰ and for April 2004, -44.52‰. No value was available for August 2004 (Fig. 3b).

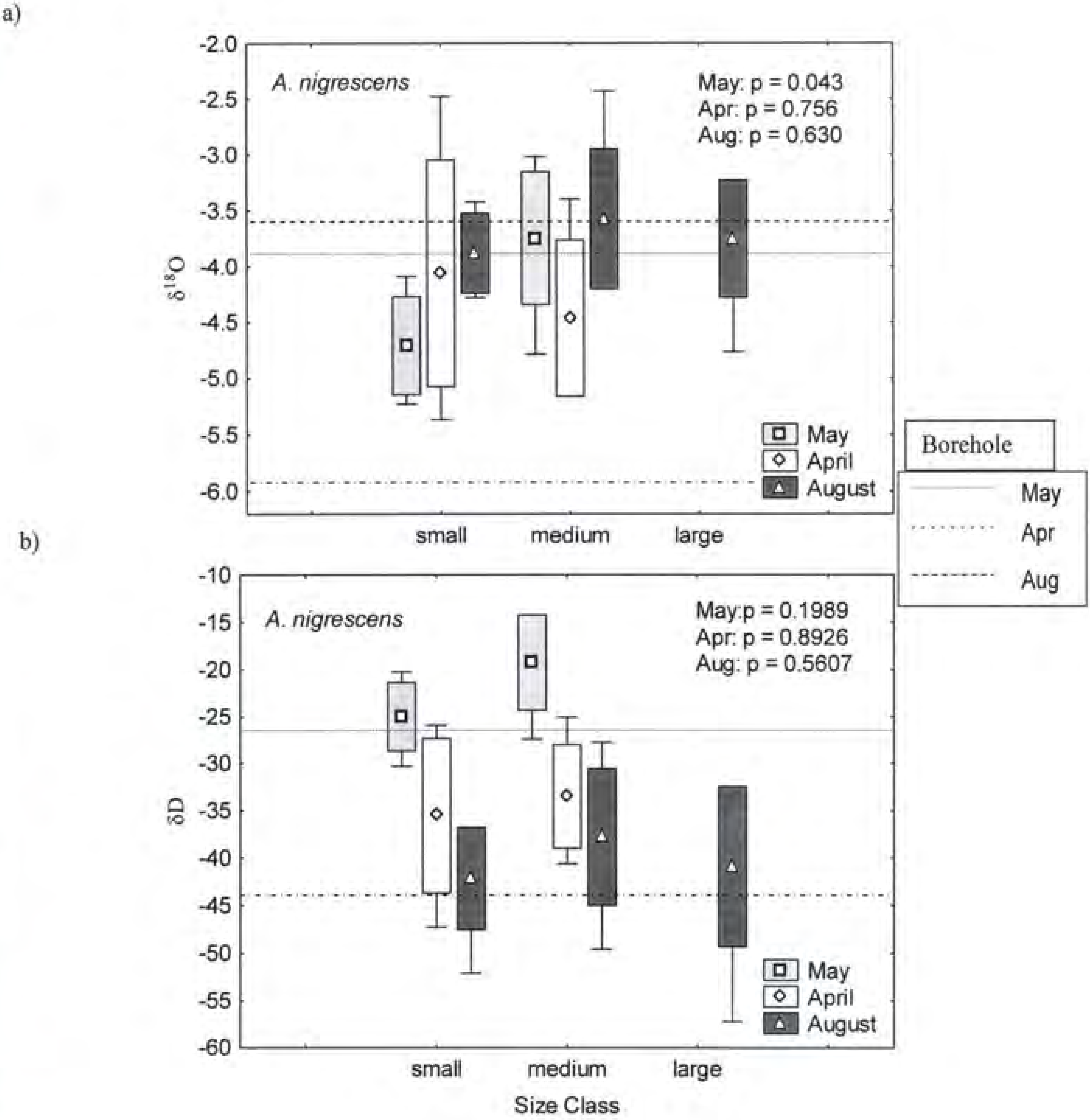

Figure 3 (a) $\delta^{18}O$ values for twigs from small, medium and large individuals of *A. nigrescens* for May 2003, April and August 2004. There was a significant difference between the small and medium size classes ($p = 0.009$) for May 2003 ($p = 0.043$), but not for any size classes in April ($p = 0.756$) or August ($p = 0.630$) 2004.
(b) The δD values for twigs from small, medium and large individuals of *A. nigrescens* for May 2003, April and August 2004. There was no significant difference between the small, medium or large size classes for May 2003 ($p = 0.1989$), April ($p = 0.8926$) or August ($p = 0.5607$) 2004.
Mean borehole isotopic values are given for May 2003 (stippled), April 2004 (dot-dash) and August 2004 (dash).

In May 2003, the medium size class twig value overlapped the May 2003 δ^{18}O borehole value, but not the small size class. In April 2004, the δ^{18}O borehole value was more negative than the mean twig δ^{18}O values for all size classes. In August 2004, all size classes' mean δ^{18} O overlap with the mean δ^{18}O August borehole value (Fig. 3a). For May, the δD values of the small individuals overlap with the mean δD borehole value, but the medium size class does not. In April there is only a slight overlapping between the small individuals and the April borehole δD values, whereas the medium size class does not overlap with the mean borehole δD value (Fig. 3b). It is unclear why the April δ^{18}O borehole value is so different to the May and August δ^{18}O borehole values. All size classes of trees apart from May small (Fig. 3a) had δ^{18} O isotopic signals that overlapped the May and August δ^{18} O borehole values, which could suggest that the trees are sourcing ground water.

4.3.3. *Terminalia sericea*: between size classes

The δ^{18}O values for *T. sericea* are significantly different between small and medium ($p=0.009$) and the medium and large ($p=0.043$) and small and large ($p=0.000$) for May 2003 ($p = 0.0001$) (Fig. 4a). In August there was a significant difference between the medium and large size classes ($p = 0.032$). There was no significant difference between the size classes for April (Fig. 4a).

For δD there was a significant difference between the small and large size classes for May ($p= 0.001$) and between the medium and large size classes in August ($p=0.029$) (Fig. 4b). There were no significant differences between size classes in April ($p=0.228$).

4.3.4. *Terminalia sericea*: borehole

For δ^{18}O, none of the May size classes overlapped with the mean May δ^{18}O borehole value (Fig. 4a). The same is true for April 2004 and August 2004 for all size classes

(Fig. 4a). However the May 2003 and April 2004 values for $\delta^{18}O$ are similar between size classes and the respective mean borehole value (Fig. 4a).

None of the May δD values for any of the size classes overlapped with the mean borehole δD value (Fig. 4b). For April the small, medium and large size classes overlap with the mean borehole value for δD. No values were available to compare for August 2004.

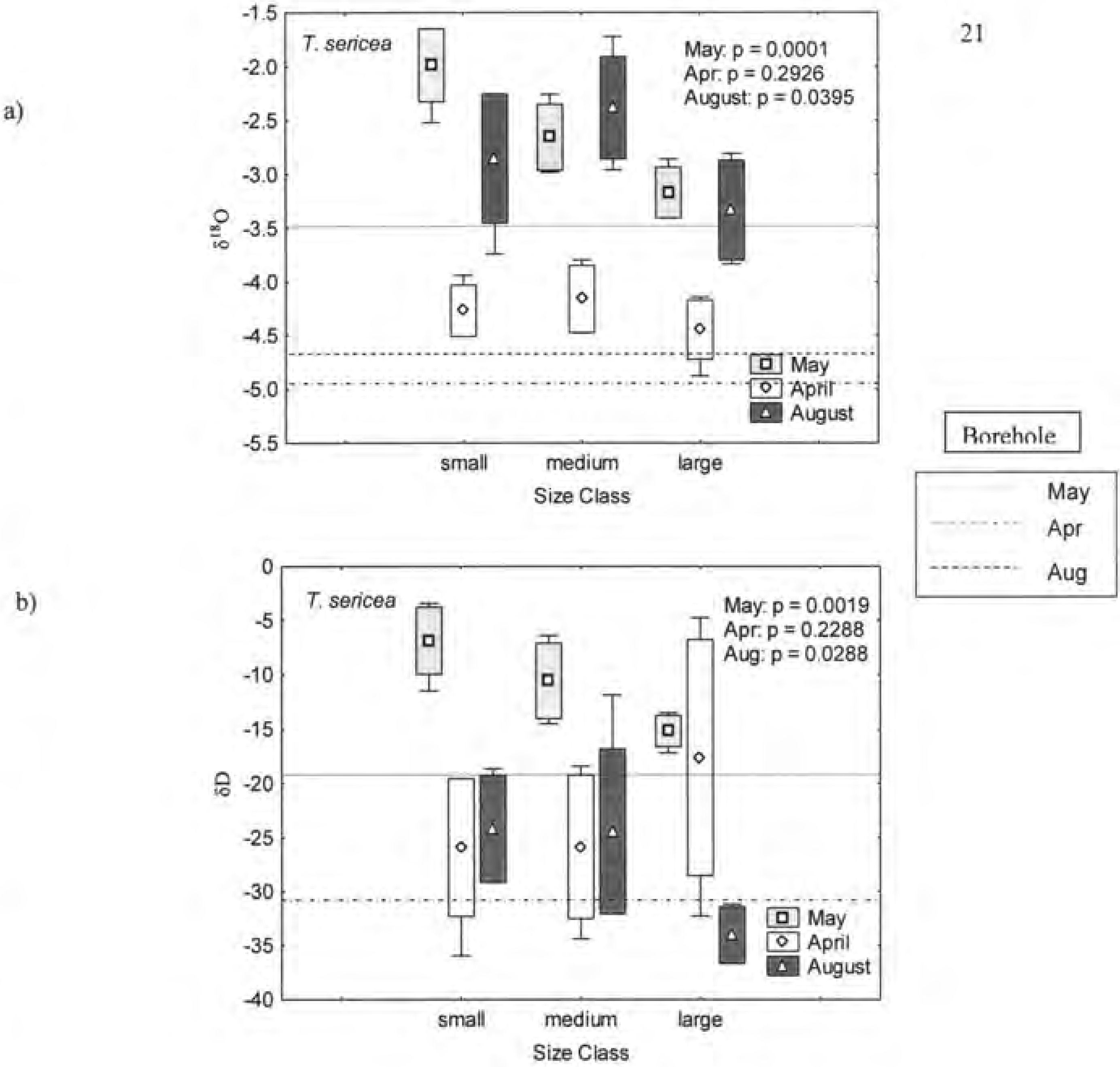

Figure 4 (a) The $\delta^{18}O$ values for twigs from small, medium and large individuals of *T. sericea* for May 2003, April and August 2004. There was a significant difference between all the size classes (small and medium: p = 0.009; small and large: p = 0.000; medium and large: p = 0.043) for May 2003 (p = 0.001) and between the medium and large size classes (p = 0.032) for August 2004 (p = 0.0395). No significant differences between size classes were observed for April 2004 (p = 0.2926).
(b) The δD values for twigs from small, medium and large individuals of *T. sericea* for May 2003, April and August 2004. There was a significant difference between the small and large size classes (p = 0.001) in May 2004 (p = 0.0019) and between the medium and large size classes (p = 0.046) in August 2004 (p = 0.0288). No difference between size classes were observed for April 2004 (p = 0.2288).
Mean borehole isotopic values are given for May 2003 (stippled), April 2004 (dot-dash) and August 2004 (dash).

4.4. Stable Carbon isotopes

T. sericea $\delta^{13}C$ values show no significant difference between the means for small medium or large individuals ($p = 0.7890$) (Fig. 5). Spread around the mean is greatest for the small and large size classes.

A. *nigrescens* shows a similar trend, where there is no significant difference between the mean $\delta^{13}C$ values of any of the size classes ($p = 0.9743$). An unquantified trend is the increase in the variance around the mean a size class increases (Fig. 5).

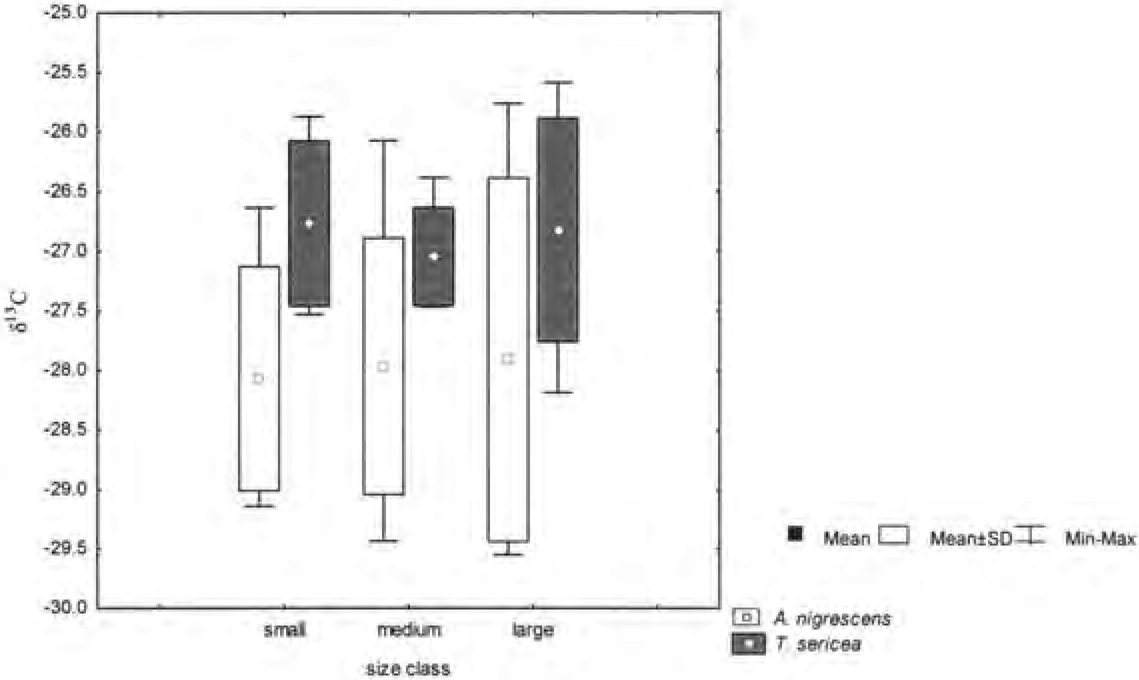

Figure 5: Distribution of $\delta^{13}C$ values between different size classes. *T. sericea* shows no significant difference around the mean for small, medium or large individuals ($p = 0.7890$). There is no difference between the mean $\delta^{13}C$ values for *A. nigrescens* for any of the size classes ($p = 0.9743$).

4.5. Water stress

4.5.1. Acacia nigrescens

For April, both size classes showed a significant increase in xylem pressure potential from morning to midday (Fig. 6a). There was a significant difference between midday and predawn pressure potential for both small ($p= 0.000$) and medium ($p= 0.005$) size classes. Furthermore, both size classes experienced equal change in stress from predawn to midday; there was no significant difference for predawn ($p = 0.93$) or midday ($p=0.84$) between size classes.

Within size classes midday pressure potential were significantly higher than predawn pressure potentials for August (small: $p = 0.0076$, medium: $p = 0.000$, large: $p= 0.000$) (Fig. 6b). There was no significant difference for predawn ($p = 0.08$) pressure potentials between all size classes whereas midday pressure potentials increased significantly ($p = 0.03$) with size class. The large size class had significantly higher xylem pressure potentials than the small size class ($p = 0.041$) (Fig. 6b).

Between size classes there was no difference between the differential pressure potentials (midday-predawn) for April ($p= 0.9464$) or August ($p=0.1610$) (Fig. 6c). Within size classes, medium ($p= 0.003$), but not small ($p=0.^{18}4$) were significantly different between April and August.

4.5.2. Terminalia sericea

For April there is a tendency ($p = 0.078$) of increasing predawn pressure potentials from small to large (Fig. 7a). In August there is a similar significant trend ($p= 0.0338$) for increased predawn pressure potentials with increasing size (Fig. 7b). This pattern does not hold for the midday pressure potentials ($p = 0.3$). All size classes has significantly higher pressure potentials in the afternoon compared to the predawn (small $p= 0.000$, medium: $p = 0.000$, large $p = 0.000$). All size classes showed a similar increase from predawn to midday pressure potentials ($p =0.431$) as shown by the differential graph (Fig. 7c).

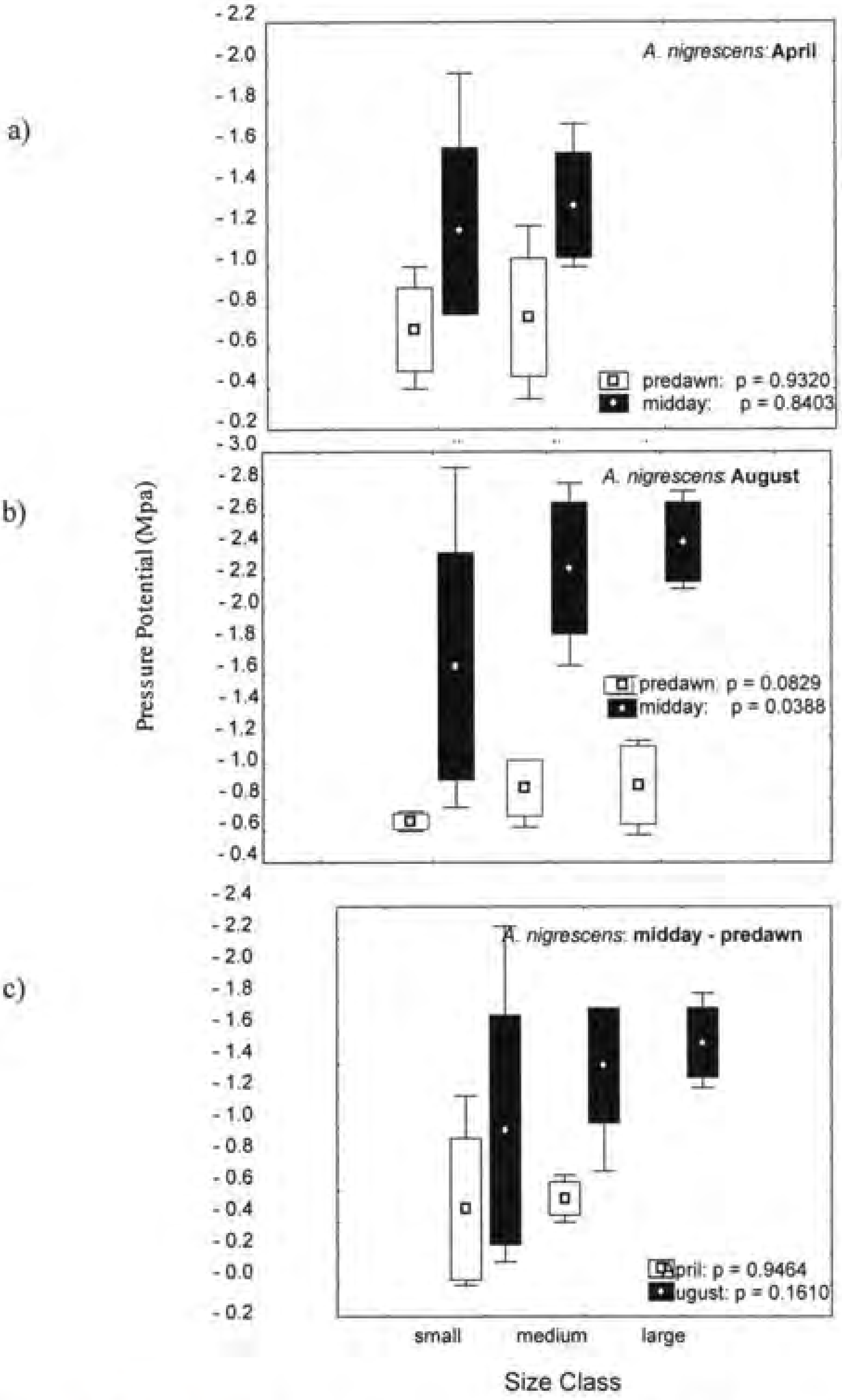

Figure 6: Predawn and midday pressure potential values for the different size classes of *A. nigrescens* for a) April 2004, b) August 2004 and c) the difference between midday and predawn readings for April and August 2004. There was no significant difference in predawn (p = 0.932) or midday (p = 0.8403) pressure potential readings between small and medium size classes in April (a). In August, midday pressure potentials were significantly different between small and large size classes (p = 0.041) (b) There was no difference between classes for predawn pressure potentials (p = 0.0829). Midday pressure potentials were consistently significantly higher than predawn pressure potentials for all size classes in April and August. No significant difference in midday-predawn pressure potentials were observed between size classes for April (p = 0.9464) or August (p = 0.161) (c).

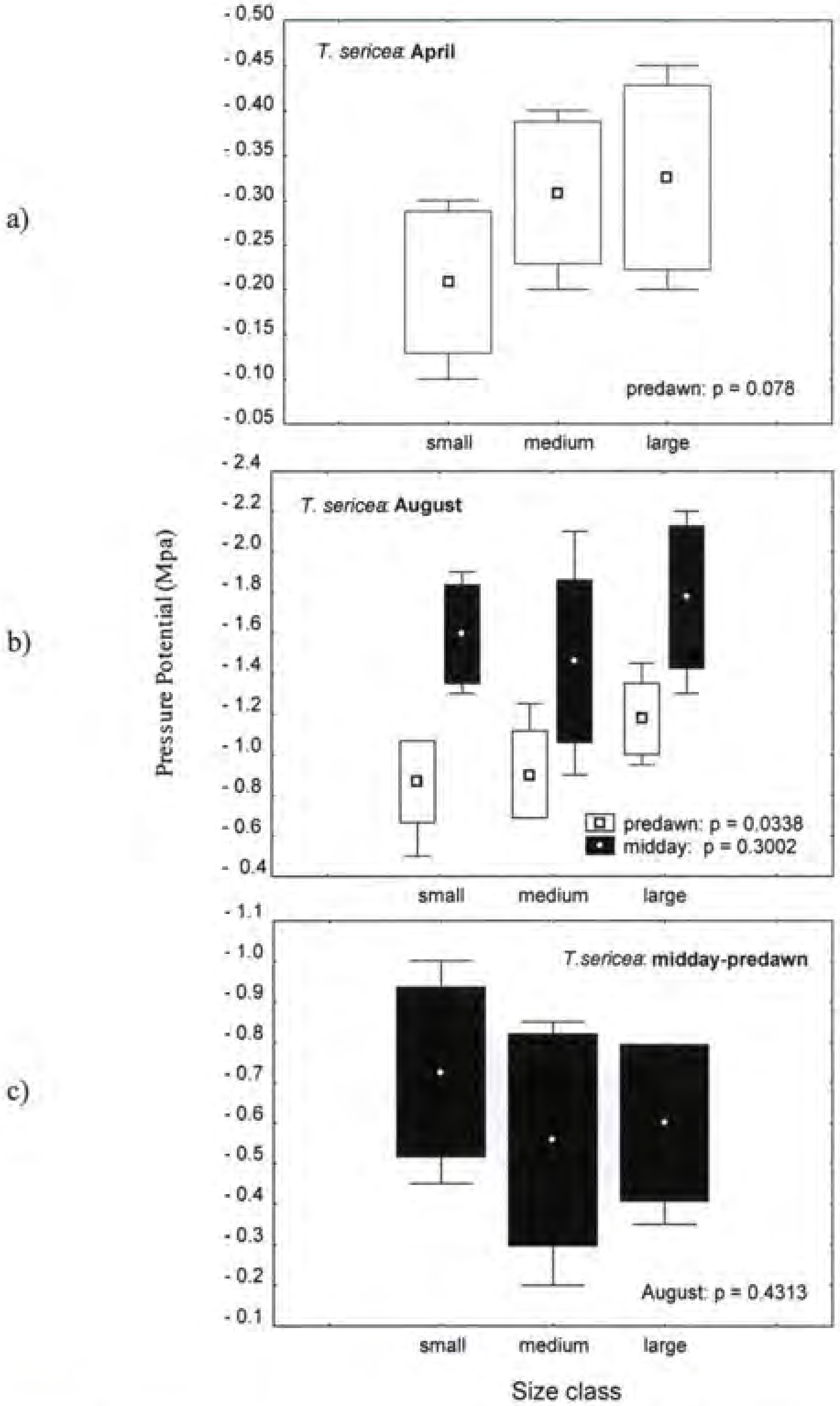

Figure 7: Predawn and midday pressure potential values for the different size classes of *T. sericea* for a) April 2004, b) August 2004 and c) the difference between midday and predawn readings for April and August 2004. There was no significant difference in predawn ($p = 0.078$) pressure potential readings between size classes in April (a). In August, predawn ($p = 0.0338$) but not midday ($p = 0.3002$) pressure potentials were significantly different between size classes (b). No significant difference in midday-predawn pressure potentials were observed between size classes for August ($p = 0.4313$) (c).

4.5.3. Between species

In April the predawn pressure potential readings are significantly higher for A. *nigrescens* (0.7MPa) than for *T. sericea* (O.2MPa) for small (p=0.000) and medium (p=0.005) size classes (Fig. 8a). In August the predawn pressure potential values for both species grouped closer together with *T. sericea* individuals of all size classes showing increased stress (Fig. 8b). Small (p = 0.036) and large (p = 0.047) individuals of *T. sericea* had significantly greater August predawn pressure potentials than small and large *A. nigrescens* individuals (Fig. 8b).

Small *A. nigrescens* individuals show high variation in August midday pressure potentials which decreases from medium to large size classes (Fig. 8c).

Comparing size classes between the species showed no significant difference between the means of the small (p = 0.875) but significantly higher august midday pressure potential values for medium (p = 0.007) and large (0.004) *A. nigrescens* size classes.

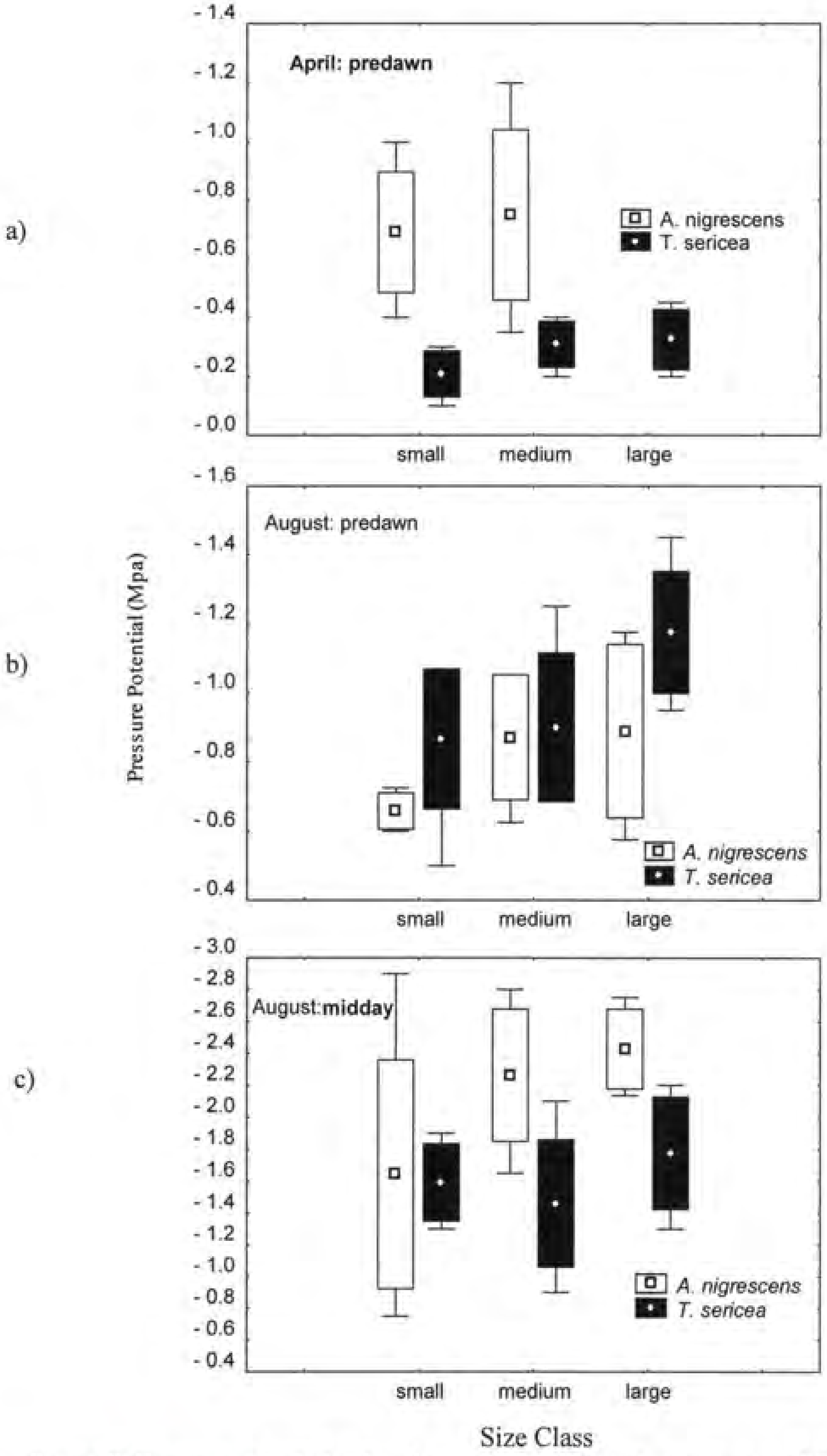

Figure 8: Pressure potential values between the different size classes of *T. sericea* and *A. nigrescens* for a) predawn April 2004, b) predawn August 2004 and c) midday August 2004. (a) There was a significant difference between the small (p = 0.000) and medium (p = 0.005) for April predawn. (b) For August predawn the small (p = 0.036) and large (p = 0.047) sizes were significantly different between species, but not for the medium (p = 0.806) size class (c). For August midday the medium (p = 0.007) and large (p = 0.004) size classes were significantly different between species, but not the small (p = 0.875) size classes.

5. Discussion

This analysis asks whether tree sizes classes use different sources. In addition I attempt to relate utilisation of a particular water source can be related to plant water stress.

The only difference in water use by different size classes is in May 2004, where the small size class of *A. nigrescens* is using water with a $\delta^{18}O$ value that is significantly ($p = 0.009$) less negative than the value for the medium size class; the same trend is prevalent in δD, but is not significant. The rest of the $\delta^{18}O$ and δD results suggest that all size classes of *A. nigrescens* are utilising the same water source (Fig. 3).

It is difficult to determine what water source *A. nigrescens* trees are utilising. $\delta^{18}O$ values may suggest that all size classes (except for May small) (Fig. 3a) are utilising water with a similar signature to August and April borehole water. Average stem $\delta^{18}O$ values also lie closer to average $\delta^{18}O$ borehole values on the local meteoric water line (Fig. 2).

Stable carbon isotope analysis (Fig. 5) for *A. nigrescens*, taken in August, revealed that small size classes did not experience significantly more seasonal water stress over the previous season compared to medium or large size classes ($p = 0.97$). This is further confirmed by the April xylem pressure potentials that indicate no significant differences in water stress between any of the size classes (Fig. 6a). April measurements were made at the end of the rainy season when there was still a lot of soil moisture available, yet dry month (August) xylem pressure potentials also did not indicate small trees to be more water stressed (Fig. 6b and c). Towards the middle of the dry season (August), xylem pressure potentials revealed the opposite to be true: that the large size-class was significantly more stressed than small-sized trees ($p = 0.041$). Bigger trees could experience more water stress as a result of greater transpiration due to a larger canopy size. Also, although large trees may potentially have access to more water because they potentially have more extensive root systems, the height of the tree results in increased tension on the water column (Koch *et al.*, 2004).

The $\delta^{18}O$ and $\delta^{2}D$ values for *T. sericea* indicates that when size classes are statistically different from one another, the consistent trend is for larger trees to be utilising water with a signal more closely matched to that of deep ground water (Fig. 4a and b). In May 2003, there is a gradient from small to large trees, where the medium size class seems to be utilising a water source with an intermediate composition to that of the small (less negative) and large (more negative) trees. In the other two months (April and August 2004) there is a tendency for small and medium sized trees to have similar $\delta^{18}O$ and $\delta^{2}D$ values, with the large trees most consistently accessing water from a deeper source (Fig. 4a and b).

Similar to *A. nigrescens,* stable carbon isotope analysis revealed that there was no significantly different water stress between the different size classes of *T.sericea* ($p = 0.789$) (Fig. 5). Also, in April with high soil moisture availability, there was no difference in xylem pressure potentials between size classes ($p = 0.078$), although the trend is for water stress to increase with tree size (Fig. 7a). As soil moisture becomes more deplete during the dry season, this trend becomes significant; the August values show that the large trees experience significantly more stress than the small and medium classes at predawn (Fig. 7b). At midday the trend is the same, but the result it not significant ($p = 0.3$).

It is curious that large *T. sericea* individuals have access to a deeper water source than the other size classes yet experiences greatest water stress at midday during the dry season in August. It is expected that trees with access to a more permanent water source should be less water stressed. The explanation may be again that for the taller trees the length of the water column (Koch *et al.*, 2004) together with the greater total leaf area affects the water stress the plant is under.

The meteoric water line (MWL) would show a gradient of increasingly negative δD and $\delta^{18}O$ values from rain to soil water to borehole, if rainfall is recharging the aquifer via the soil profile. This trend is seen for the Pretoriuskop MWL, but not for Satara (Fig. 2). At Satara, the soil water isotopic values separated out distinctly from both the

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borehole and rainfall values and had much more negative $\delta^{18}O$ and δD values than the boreholes (Fig. 2). This suggests that there is strong fractionation occurring within the clay soils (Fig. 2) and may also suggest that the borehole water around Satara is not primarily derived through infiltration from the soil. This is due the greater ability of clay minerals to retain water and prevent it from infiltrating the soil profile.

6. Conclusion

There is little evidence for partitioning of water resource between size classes on the basaltic clay soils at Satara. All size classes at Satara seem to be utilising water from the same source. On the sandy soils at Pretoriuskop, the small and medium tree size classes generally group together, with the large size class utilising deeper water with a signature similar to that of the boreholes. For *A. nigrescens* and *T. sericea*, the large individuals were significantly more stressed compared to the other size classes during the dry season (August 2004). This could be related to the size of the trees as it has been shown that xylem pressure potentials are increasingly negative with height above ground (Koch *et al.*, 2004).

The Walter hypothesis (1971), considers that trees and grasses exploit different layers in the soil horizon. He further predicts that that soil type will predict tree density on the basis of variable water availability at different depths in the soil. The present study on two different soil types, suggests that trees compete with grasses in the juvenile life stages. Large trees at Pretoriuskop (well drained sandy soils) access a different water source from the juveniles; the same class at Satara (poorly drained clay soils) uses the same water as juveniles. As a consequence root niche separation cannot predict tree densities across both sites, thus it cannot explain the phenomenon of tree grass coexistence of savannas.

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