Patterns of carbon allocation, storage and remobilization in a common resprouting savanna species - *Acacia karoo*

Benjamin Wigley

Supervised by Prof. W.J. Bond and Prof. M. Cramer

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B.J. Wigley

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Abstract

The aim of this study was to gain a comprehensive understanding of the patterns of carbon partitioning, storage and remobilization in *Acacia karroo* during the juvenile life history stage. Tuber total nonstructural carbohydrate (TNC) concentrations and $\delta^{13}C$ values were determined in plants from two different stages in the juvenile life history of *A. karroo*. These were one year after a fire when the plant consisted of numerous leafy shoots or coppices (coppicing stage) and three years after a fire when the plant consisted of one pole like stem (gulliver stage). Gullivers were found to have mean TNC pools of 150g and mean TNC concentrations of 33%. Coppices had mean TNC pools of 97g and TNC concentrations of 24%. Both total TNC pools and TNC concentrations in gullivers were significantly higher ($p \leq 0.05$) than in coppices. Carbon isotopes were used to determine whether growth was based on carbon reserves as heterotrophic growth shows a distinct enrichment in $\delta^{13}C$. The water relations of plants can also influence the $\delta^{13}C$ values of plant growth. However, there were no significant differences in root size and depth between the stages, indicating that all plants had access to similar water sources. Mean $\delta^{13}C$ values from the stems of plants in the gulliver stage were significantly enriched (> 1‰) in ^{13}C compared to both coppicing plants ($p < 0.01$) and adults ($p < 0.05$). The negative $\delta^{13}C$ values in coppice stems suggest that their growth is not based on stored carbon. The enriched $\delta^{13}C$ values found in the gulliver stems support the hypothesis that carbon reserves are utilized to achieve fast growth rates in an attempt to escape the fire trap. However, the small magnitude of the differences in $\delta^{13}C$ between the two stages suggests post-burn regrowth is derived from both current photosynthate and stored carbon.

Introduction

Bush encroachment has become a topic of increasing concern for scientists, landowners, and managers across the globe. This phenomenon is especially common in the savanna biomes of the world (Archer 1989; Matheson and Ringrose 1994; Moleele *et al.* 2002). It has repeatedly been shown that, in the last century or more, the dominant trend in many parts of the world has been for woody plants to increase and open vegetation to decline (Scholes and Archer 1997; Hoffmann *et al.* 1999; Bond and Midgley 2000). This process has been attributed to both climatic variability and anthropogenic interference, such as altered grazing and fire regimes (Wand *et al.* 1996). Recently, it was proposed that global increases in atmospheric carbon dioxide concentrations might be contributing to, or even driving bush encroachment (Wand *et al.* 1996; Hoffmann *et al.* 2000; Bond et al. 2003). In order to manage bush encroachment a better understanding of the belowground processes and the likely responses of the encroaching species to different management strategies are desperately needed. This is especially true in the light of changing CO_2 concentrations, since atmospheric CO_2 concentrations are known to have large effects on plant carbohydrate status. Increasing levels of CO_2 may have important implications for plant responses to fire (Hoffmann *et al.* 2000).

Savannas are broadly defined as tropical or near tropical seasonal vegetation with a continuous, often grass-dominated herbaceous layer and, in most cases, a significant but discontinuous layer of woody species (Frost *et al.* 1986). The dynamics of the tree-grass interactions that allow the co-existence of these two vegetation types have been widely studied and documented (e.g. Belsky 1994; Williams *et al.* 1996; Scholes and Archer 1997; *Higgins et al.* 2000; Bond et al 2003). The effects of fire and herbivory, especially in African savannas, are believed to be chiefly responsible for vegetation structure and for maintaining savanna-forest boundaries (Maze 2001). In many parts of South Africa *Acacia karroo* (Hayne) densities have been increasing, often to the detriment of the co-occurring grass species (Trollope 1987; O'Connor 1995; Moleele 2002). Trollope (1987) found a decrease of 46% in grass production over a six years period of severe *Acacia karroo* encroachment in the Eastern Cape, South Africa. A number of other authors (e.g. O'Connor 1995; Chirara *et al.* 1998; Skowno *et al.* 1999; Moleele *et al.* 2002) have reported *Acacia karroo* as a major contributor to bush encroachment in Southern African savannas. One such area is the Hluhluwe-Umfolozi Park, KwaZulu Natal, South Africa,

which has experienced major bush encroachment in the recent past (Whateley and Porter 1983; Balfour and Howison 2001). One of the major encroaching species in this area is *Acacia karroo*.

A common attribute of most woody species occurring in areas prone to high disturbance is the ability to sprout (Bellingham and Sparrow 2000; Bond and Midgley 2003). Sprouting of woody plants is probably an early adaptive trait enabling survival after considerable damage from fire (Hodgkinson 1998). The ability of trees to sprout after frequent burning is dependent upon carbohydrate reserves, which are replenished between burns (Hoffmann *et al.* 2000). Replenishment of carbohydrate reserves following burning may require 1-2 yrs depending on the environment in which the plants are growing (Miyanishi and Kellman 1996; Kays and Canham 1991; Bell and Pate 1996; Bell *et al.* 1996). The potent resprouting ability of topkilled trees is a key life-history trait that promotes the persistence of trees in savannas (Walter 1971 cited in Maze 2001; Bond and van Wilgen 1996; Gignoux *et al.* 1997). Juveniles growing within the flame zone, termed "gullivers" by Bond and van Wilgen (1996) may persist for many years by repeatedly resprouting from ageing root systems (Bond and van Wilgen 1996). The ability to do so lies in the underground carbohydrate storage organs known as lignotubers common to most resprouting species occurring in areas prone to high fire frequency. The recruitment of these gullivers into mature size classes depends on rare escape opportunities whereby a tree attains sufficient height to escape flame damage (Bond and Midgley 2000). Changes to tree biomass or densities depend largely on the frequency of escape opportunities. In areas not exposed to heavy grazing, these are determined by long intervals between fires (Bond and Midgley 2000). However, gulliver escape is also determined by the rate at which woody plants can recover from injury and grow to escape height, thus avoiding injury from subsequent fires or canopy loss, termed top kill (Bond and Midgley 2000).

It has been shown that stable carbon isotopes can also be used as a tool to trace heterotrophic plant growth (Terwilliger and Huang 1996; Damesin and Lelarge 2003; Helle and Schleser 2004). A number of studies have found that carbohydrates (e.g. sucrose and starch) stored in plants are frequently more enriched in $\delta^{13}C$ than recently assimilated carbon (Gleixner *et al.* 1993; Terwilliger and Huang 1996; Brugnoli *et al.* 1988; Gleixner *et al.* 1998; Duranceau *et al.* 1999; Le Roux-Swarthout *et al.* 2001; Jaggi

et al. 2002; Damesin and Delarge 2003; Helle and Schleser 2004). It has been suggested that discrimination takes place during the export and/transport of sugars, probably during loading or unloading of the phloem where sucrose carriers in the plasma membrane and sometimes cell-wall invertase and cytoplasmic sucrose synthase are involved (Kuhn *et al.* 1999; Sturm and Tang 1999 both cited in Damesin and Lelarge 2003). Consequently, all organic matter incorporating these exported sugars would be enriched in $\delta^{13}C$ relative to the starting material. Damesin and Lelarge (2003) propose that the $\delta^{13}C$ of stems is altered by two fractionation steps: one during sugar transfer to the stems and one during stem respiration. Terwilliger and Huang (1996) suggested that that growth from translocated organic sources of carbon may have more enriched $\delta^{13}C$ values than growth from the intermediate products of photosynthesis, regardless of environment.

Bond and van Wilgen (1996) proposed the "gulliver strategy" as a distinct life history in savanna tree species. However, this hypothesis has not yet been adequately tested (Maze 2001). The quantitative relationships between the amounts of reserves utilized and growth initiated, or between growth and photosynthesis of new shoots and replenishment of root reserves, have not received much attention in natural ecosystems (Bowen and Pate 1993). The focus of this study is therefore to gain a better understanding of these processes in *Acacia karroo*. This species was chosen as it is thought of as a typical species that adopts the gulliver strategy and it is one of the major encroaching species in many parts of Southern Africa. This study uses two main techniques in order to gain a better understanding of the patterns of carbon partitioning in *Acacia karroo* during two different stages within the juvenile life-history stage. When resprouting after a fire *A. karroo* sends out numerous coppice shoots, referred to as the coppicing stage in this study. Bond and Maze (unpublished) propose that once some threshold size has been reached, a marked asymmetry develops among resprouting stems. One stem usually grows taller than the others, forming a pole like stem and eventually the shorter coppices die. This second stage within the juvenile stage of *A. karroo* has been referred to as the gulliver stage. The main objective of this study is to determine whether plants store carbon in the roots to maintain regrowth after a fire and which plant organs depend more on stored carbon. The hypothesis being that the pole stage of gullivers is more dependent on stored carbon because this is the stage on which reproductive benefits more depend (Fig 1).

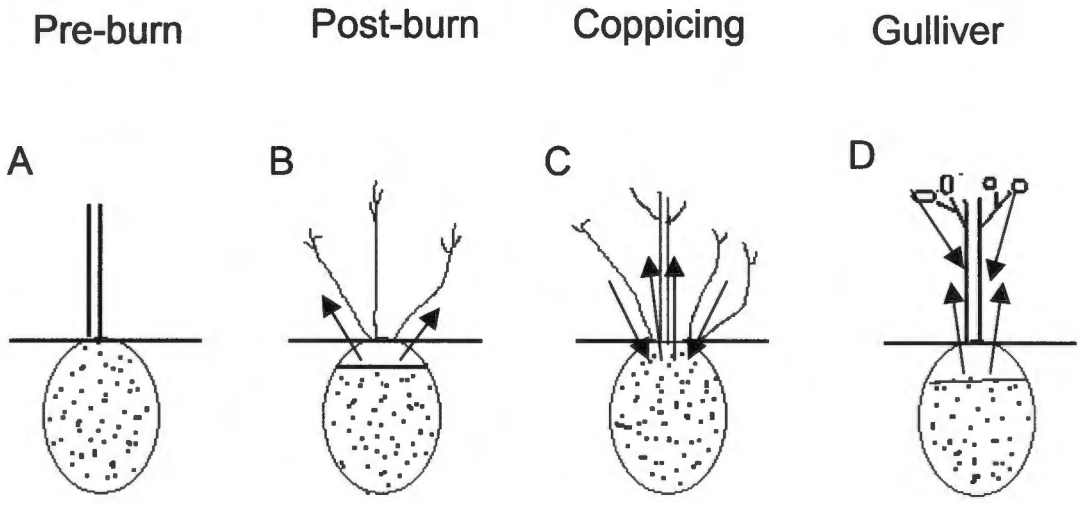

Fig.1. A schematic diagram of starch allocation to post-fire organs. A) Gulliver tubers with high starch content. B) Carbon reserves are used to resprout after a fire. C) The coppices recharge the carbon reserves and one stem begins to outgrow the other coppices. D) The coppices die away and the main stem grows tall by using both current photosynthates and stored carbon.

Methods

Study site

The study was undertaken in the Hluhluwe-Umfolozi Park in KwaZulu Natal, South Africa. The chosen study sites (Fig 2) were selected off the Nyalazi road in the corridor section of the park (S 28.26963 E 31.94231). The area has an elevation of approximately 273m above sea level with an average annual rainfall of approximately 840mm for the previous three years. The long-term average annual rainfall for the elevation of 273m is 780mm, calculated from the equation provided by Balfour and Howison (2001).

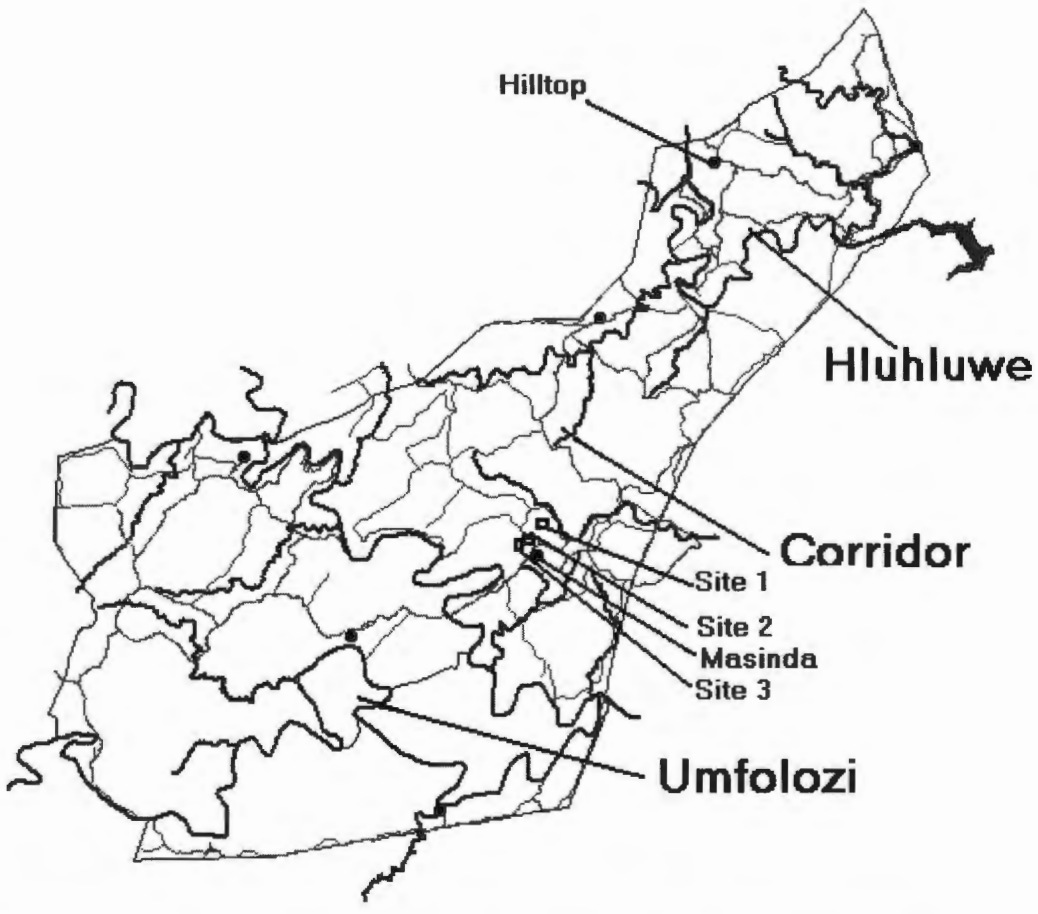

Fig.2. Map of the Hluhluwe-Umfolozi Park, Northern Kwazulu-Natal. Sites 1,2&3 correspond to the gulliver, coppice and adult sites consecutively.

For this study, *Acacia karroo* plants in three different stages of its life history were selected. The first site (hereafter referred to as the "gulliver site") was in an area that burnt three years prior to the study (28th Aug 2001) ensuring that many of the *Acacia karroo* juveniles were in the gulliver stage. The second site (hereafter referred to as the "coppice site") was selected in an area that burnt one year prior to this study (8th Aug 2003) ensuring that all plants were in a coppicing stage. The third site (hereafter referred to as the adult site) occurred a little further down the valley below site two in an area surrounded by large boulders, allowing the plants protection from fire, thereby allowing them to reach adult status. The sites were close enough to each other (<1km) to assume similar rainfall and soil type.

Data collection

At the gulliver site, ten *A. karroo* plants were selected. For each of the plants the total height and basal diameter were measured and recorded. The plants were excavated ensuring recovery of the top 50cm of the main root. Roots recovered were put on ice and frozen on return from the field. A branch representing the last years growth was collected from the canopy of each plant. The pole like stem of each plant was also recovered for starch and isotope analyses. At the coppice site ten plants were selected. For each of these plants the height and diameter of the top killed stem was measured. All of the coppice shoots were clipped at ground level and taken for analysis. The top 50cm of the main root was excavated and taken for analysis. At the adult site, five *A. karroo* plants of similar size were selected. Each plant was felled and a disc section was taken from the stem at a height of approximately 1m from the base.

Tissue analysis

The pole-like stems of the gullivers were chopped into 15cm sections with a 2cm thick disc taken at each 15cm interval. The root material was sawn into approximately 2cm thick discs and these discs were then chopped into small bits. The sawdust from each root was collected for starch analysis. All plant material was dried in an oven to constant dry weight (ca 72-96h) at 80 C. The dry weight of total coppice material and of each root was measured and recorded.

Stable carbon isotope analysis

For each of the gullivers a stem section taken at ground level was used for isotope analysis. A 1mm hole was drilled at every mm along the radius of each disc starting at the centre and working outwards towards the edge of the disc. For each hole drilled, the shavings created from drilling were carefully collected ensuring no contamination by other sawdust. The above procedure was repeated on the discs taken from the base of the branch taken from the canopy of the gullivers, on the discs taken from the base of the thickest coppice and on the discs taken from the adult plants. A sub-sample of the sawdust taken from each root was milled in a ball mill for isotope analysis. From each wood sample a 60 to 80µg sub sample was weighed and combusted in an elemental analyzer (Model NA 1500; Carlo Erba, Milan Italy). Carbon isotope ratios of the generated CO_2 were determined using a Finigan Mat 252 Mass Spectrometer. $\delta^{13}C$ was

referred to the international standard VPDB (Vienna Pee Dee Belemnite): $\delta^{13}C$ (‰) = $(R_S/R_{VPDB} -1) \times 1000$, where R_S and R_{VPDB} are the molecular abundance ratios of carbon isotopes, $^{13}C/^{12}C$, of the sample and the standard VPDB, respectively.

Starch analysis

Material used for starch analysis was finely ground and oven dried at 80 C. Starch concentrations in the tubers of the gullivers and coppicing plants, and in the stems of the gullivers were determined. The colorimetric method described by Buysse and Merckx (1993) were used for the starch analyses. Samples of 0.05g of ground plant material were hydrolysed in a 3%HCl solution (5ml) in a boiling water bath for three hours. The hydrolysis products were centrifuged, the supernatant decanted and made up to 50ml with 80%ethanol. Because sugar concentrations in this solution yielded absorbance readings above the linear portion of the standard curve an additional 1/5 dilution in 80% ethanol was necessary. The samples, containing 150µl of sugar solution, 150µl of phenol solution (28%w/w, diluted in 80% ethanol) and 750µl of concentrated H_2SO_4 were allowed to stand for 15 minutes before their absorbences were measured at 490nm, using a spectrophotometer. Absorbences were converted to concentrations using starch and glucose standards. A number of replicate measurements and internal standards were used in order to ensure consistent results.

Data analysis

The data were analyzed using Statistica 6. Variables used in analyses were tested for homogeneity of variance in order to determine if parametric or nonparametric tests were needed. If raw data were not normally distributed, they were log-transformed then tested for normality. One-way ANOVA analyses were used if the requirements for normality were met. When these conditions were not met, Kruskal Wallace and Mann-Whitney U-tests were performed on the data.

Results

There was found to be no difference in either mean size or depth of the rooting systems in the gulliver versus the coppicing stage. The adult plants in this study were not excavated and were likely to have had deeper rooting systems than the other stages. An attempt was made to age the adults using tree rings; this however was not possible due to the lack of

clear growth patterns in the stem. Thus, it was not possible to align the $\delta^{13}C$ sequences with the corresponding rainfall during their time of formation.

The average annual rainfall for the previous three years (Fig.3) shows years 2001 and 2002 to be dry years, while 2003 was a relatively wet year, well above the long-term average for the area. The graph also shows that the rainfall at Hluhluwe was consistently higher than at Masinda, presumably because Hluhluwe is at a higher altitude.

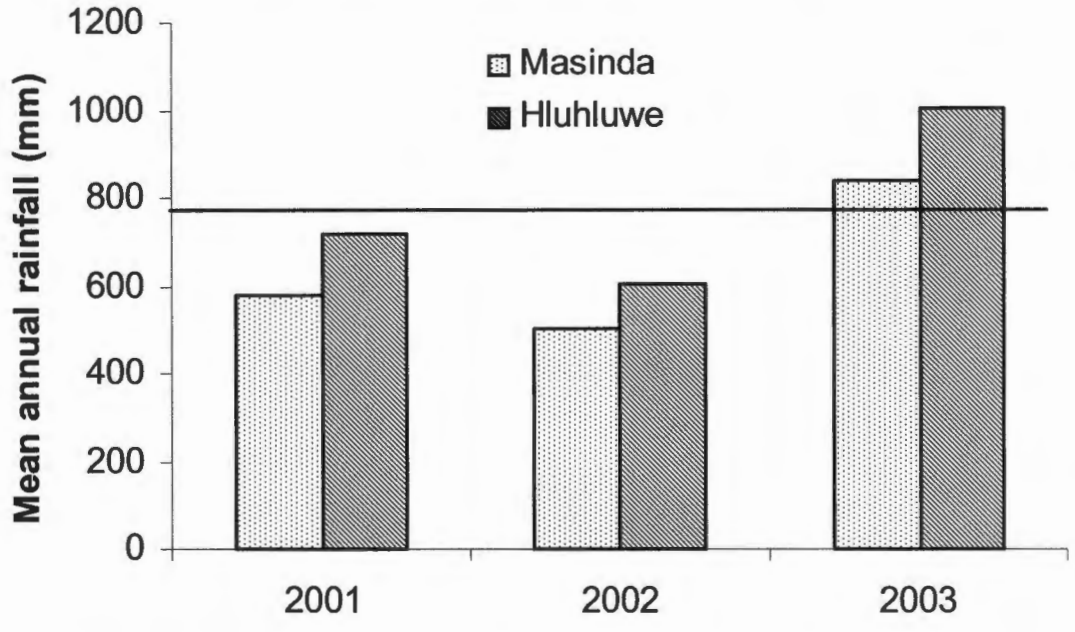

Fig.3. Average annual rainfall for the previous three years at the study site. The data for Masinda were provided by KZN wildlife, data for Hluhluwe were provided by the SAWS. The average annual rainfall for the altitude at the study site (780 mm), provided by Balfour and Howison (2001) has been included.

There were no differences in the size and dry weight (Figs.4-5 & Table1) of tubers from the two different life history stages, i.e. gullivers compared to coppicing plants (One-Way ANOVA, $p>0.4$, $df = 18$). The gullivers had significantly larger stem diameters than the coppicing plants, (mean gulliver = 3.1 cm, mean coppice = 2.1 cm, One-Way ANOVA, $p<0.001$, $df = 18$). The starch concentrations (Fig. 6) of the gulliver tubers were significantly higher than coppice tubers (Fisher LSD test $p < 0.05$) and gulliver stems ($p < 0.01$). There were no significant differences between starch concentrations in coppice tubers and gulliver stems. Plants in the gulliver stage had significantly higher total root TNC content than plants in the coppicing stage (Fig. 7& Table 1).

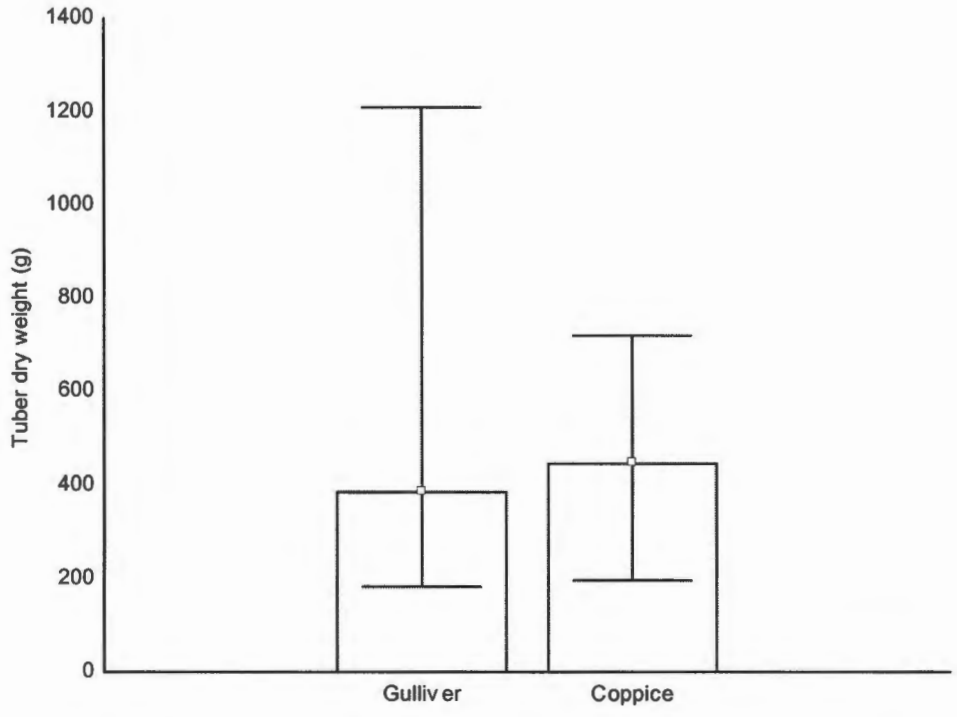

Fig.4. Median tuber dry weights for the two life stages with whiskers showing the range of dry weights found for each group (n = 10 in each group). There were no statistically significant differences in dry weight between the two stages.

Table 1. Mean, median and standard error of tuber dry weights, starch concentrations, and total starch pools of plants in the two stages (n = 10 for each group).

	Gulliver mean	Coppice mean	Gulliver median	Coppice median	Gulliver std error	Coppice std error
Tuber dry weight (g)	538	433	386	446	110	60
Starch concentration (%)	32.8	23.7	34.4	21.7	4.0	2.1
Starch pool (g)	150	97	138	90	19	12

Fig. 5. Photographs of the smallest (A) and biggest (B) tubers from plants in the gulliver stage and the smallest (C) and biggest (D) tubers taken from the coppicing plants. The ruler in the picture is 15cm in length. Note the similarity in both size and morphology of gulliver and coppice roots.

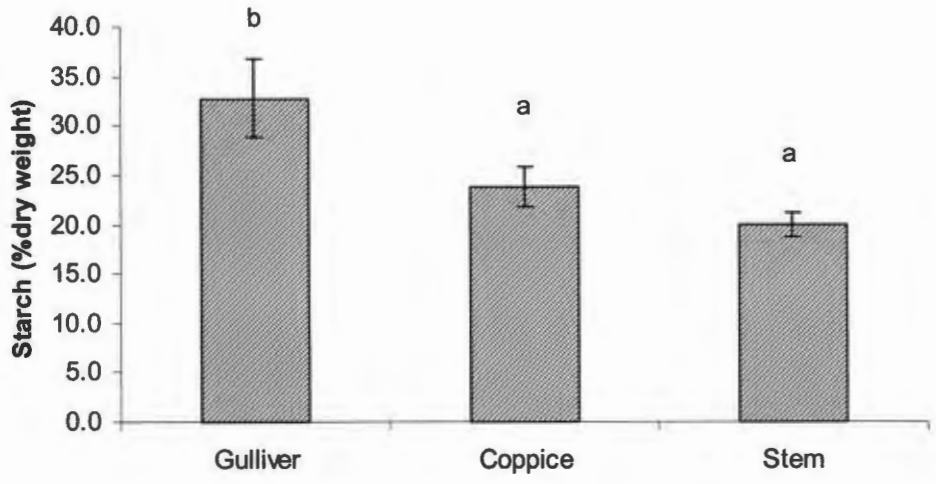

Fig.6. Mean starch concentrations (± SE) of plants in the gulliver and coppicing stages. Stem starch concentrations were only measured from plants in the gulliver stage. There were no significant differences between (a) while differences between (a) and (b) were significant (Fisher LSD test, $p < 0.05$).

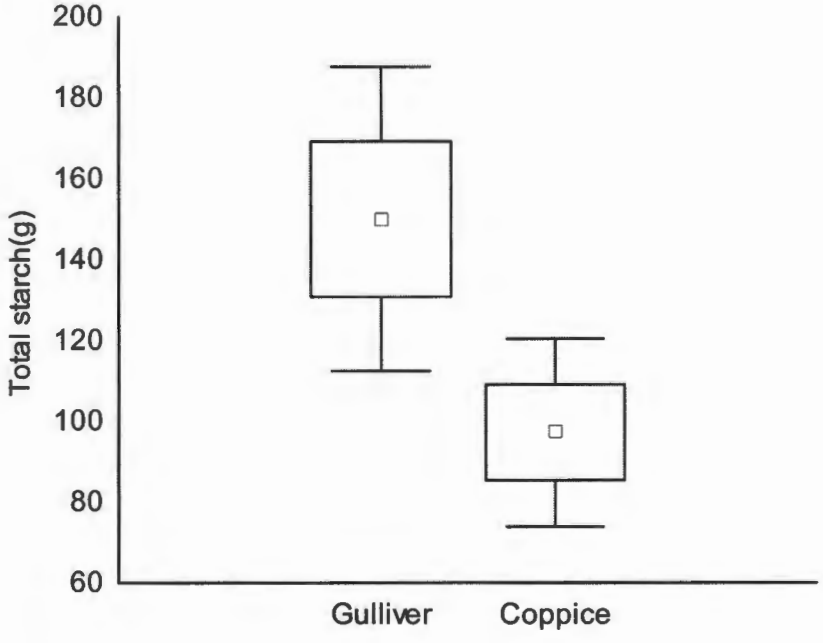

Fig.7. Mean total starch pools (g) in the tubers of plants in the gulliver and coppicing stages. Differences between the two stages were significant (Students t-test, $p < 0.05$). Means ± SE (box) and 95% confidence intervals (whiskers) are shown.

There was a highly significant correlation between stem diameter and plant height (Table 2). No correlation was found between tuber dry weight and plant height.

However, a significant correlation was found between starch concentrations and height as well as total starch and height. Stem diameter was also found to correlate to the total starch pools in tubers. Tuber dry weight was negatively correlated to starch concentrations and tuber dry weight was highly correlated to total starch pools in tubers.

Table.2. A correlation matrix for the measurements performed on all plants. Plant height and stem diameters were measured on live gullivers, and on the skeletons of the top-killed coppicing plants (n = 10). The upper numbers in each row are the correlation coefficients (r), * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant.

	stem diameter (at 5cm)	tuber dry weight (g)	% starch	total starch (g)
plant height	0.78	0.21	0.44	0.59
	***	NS	*	**
stem diameter (at 5cm)		0.33	0.25	0.55
		NS	NS	**
tuber dry weight (g)			-0.47	0.76
			*	***
% starch				0.12
				NS

The $\delta^{13}C$ values across the radius of the gulliver stems showed a clear pattern with relatively positive values (-26.5 ‰ $\delta^{13}C$) at the centre of the stem decreasing by *ca.* 1‰ up to 4 mm from the centre and then increasing up to *ca.* 8 mm from the centre before decreasing towards the periphery by ca. 2-3‰ (Fig.8A). The mean $\delta^{13}C$ values of the coppices showed slight enrichment (0.5‰) between 0 and 1 mm from the centre then decreased steadily towards the periphery to reach a final value of *ca.* -27.5 ‰ $\delta^{13}C$ (Fig8B). Mean $\delta^{13}C$ values of the branches taken from gulliver canopies were consistently depleted in $\delta^{13}C$ (*ca.* -27.5 ‰, Fig.8C). Mean $\delta^{13}C$ values from adult stems were relatively constant (between -27.5 and -26.5 ‰, Fig.8D) across the entire stem cross section.

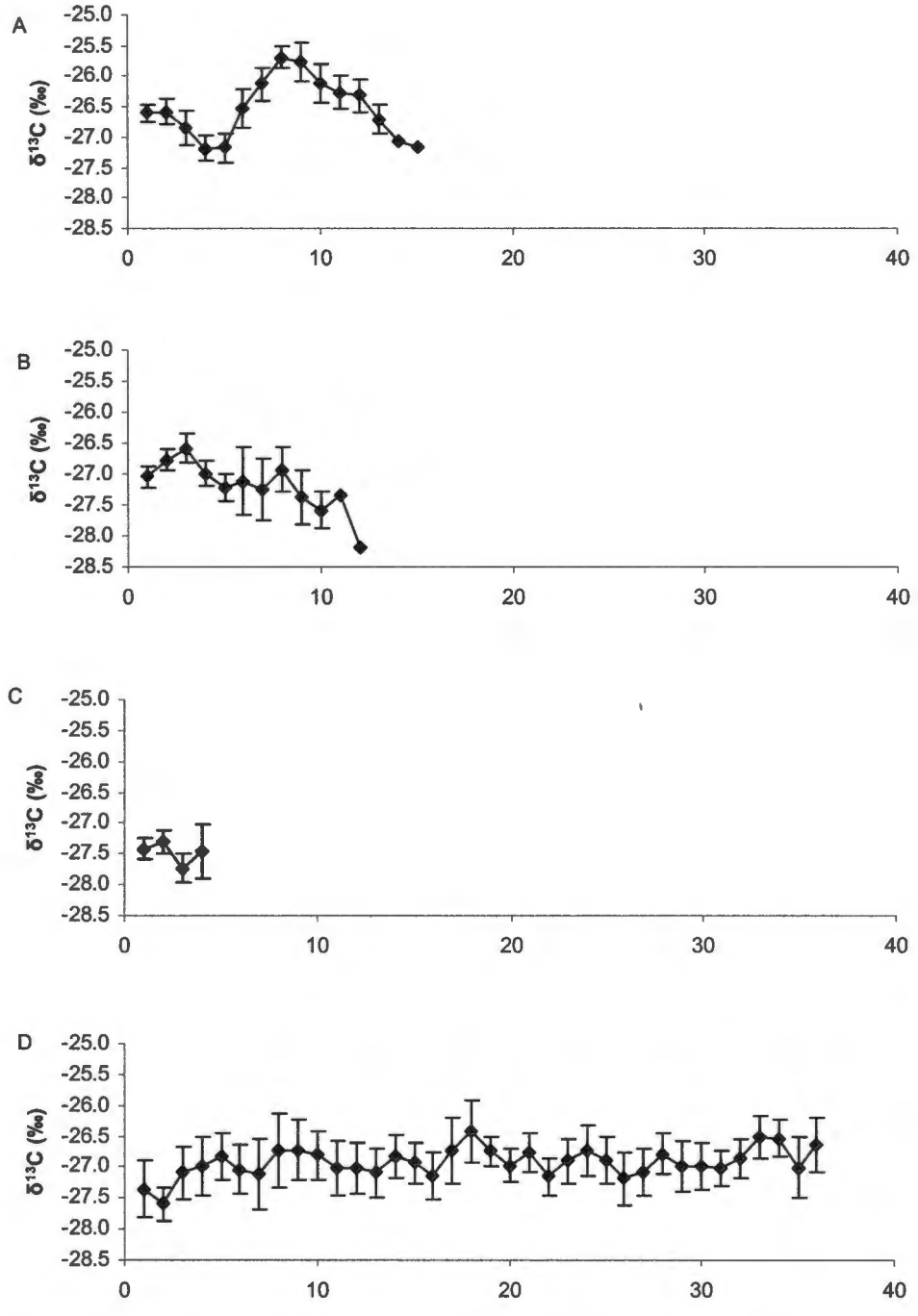

Fig.8. $\delta^{13}C$ values (mean ± SE) moving from the centre of the stem cross section (distance = 0 mm) for (A) gulliver stems (n = 10); (B) coppice stems (n = 10); (C) branches taken from the gulliver canopies (n = 10); (D) adult stems (n = 5). Samples were then taken at 1 mm intervals along the radius of the stem.

The $\delta^{13}C$ values of each life history stage (coppicing, gulliver and adult) were compared by combining the values of all plant parts sampled. The nonparametric Kruskal-Wallace test showed significant differences between stages. Multiple comparisons between groups showed significant differences in $\delta^{13}C$ between the gullivers

and coppicing plants and between the gullivers and adults. However, there was no difference in $\delta^{13}C$ values between the coppicing plants and adults. The gullivers can be seen to have the most positive $\delta^{13}C$ values (Fig.9).

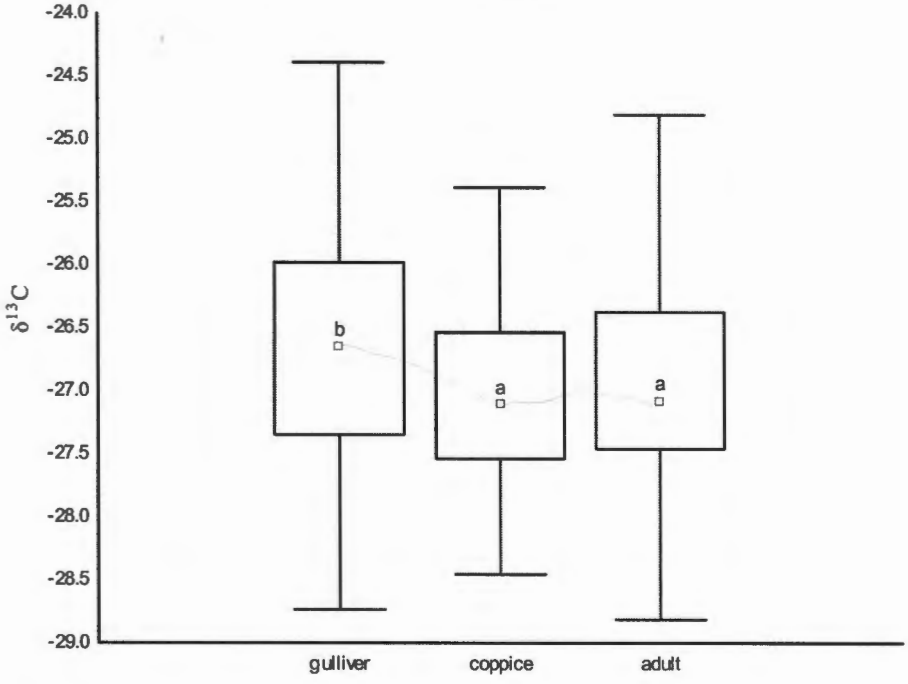

Fig. 9. Median $\delta^{13}C$ values for each of the three life history stages sampled. Values include all plant parts sampled for each stage. Kruskal-Wallis test: H (2, N= 417) =10.47 p =0.0053). Differences between (a) are not significant while differences between (a) and (b) are significant at ($p < 0.05$). Medians, 25%-75% quartiles (box) and ranges (whiskers) are shown.

The different plant tissues sampled for each of the above groups were then separated and compared. The assumption of equal variances was met for these comparisons (Levene Test of Homogeneity of Variances, $p > 0.05$). Significant differences were found between means. The Tukey HSD for unequal N (Spjotvoll/Stoline) Post-hoc test showed significant differences in $\delta^{13}C$ values between gulliver stems and all comparisons except gulliver roots and coppice roots. All other differences in $\delta^{13}C$ were not significant. When comparing each plant part sampled within each stage (Fig.10), the gulliver stems were over 0.5 ‰ more positive than all other plant parts. The next most positive $\delta^{13}C$ values were found in the adult stems which were very similar to both gulliver roots and coppice stems. The coppice roots and gulliver branches had the most negative $\delta^{13}C$ values.

Figure 11 shows the $\delta^{13}C$ values for the growth of gulliver stems compared with canopy branches growing over the same period. The growth was aligned by looking at

growth patterns from the stem cross sections. The values from the gulliver stems were found to be significantly more positive than those of the branches.

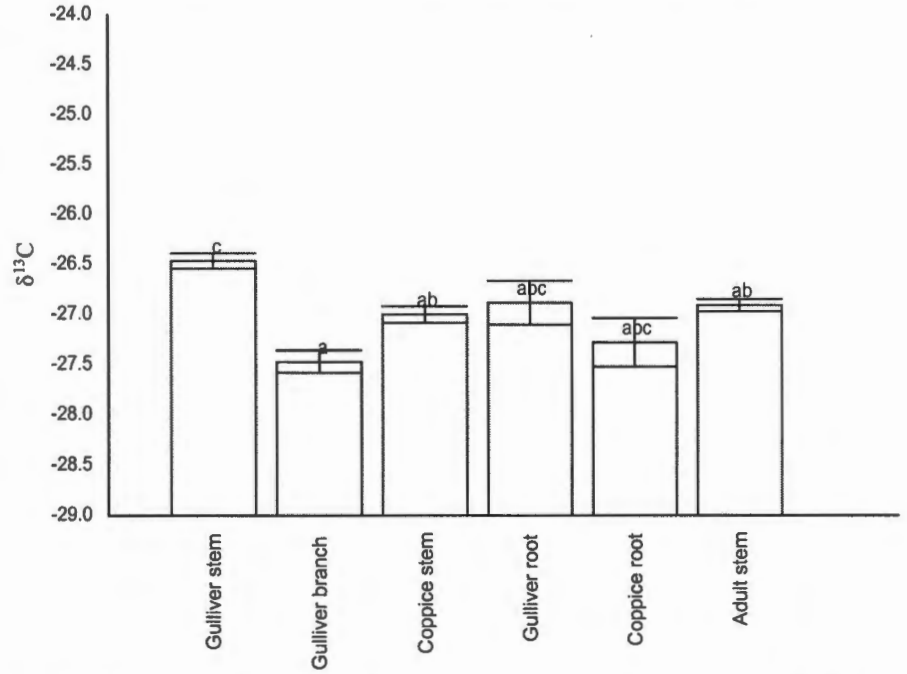

Fig.10. Mean ± SE $\delta^{13}C$ values for each plant part sampled in each of the three life-history stages. Significant differences were found between means (One-Way ANOVA, $p<0.0001$, $df = 410$). Differences between letters are all significant at $p < 0.01$.

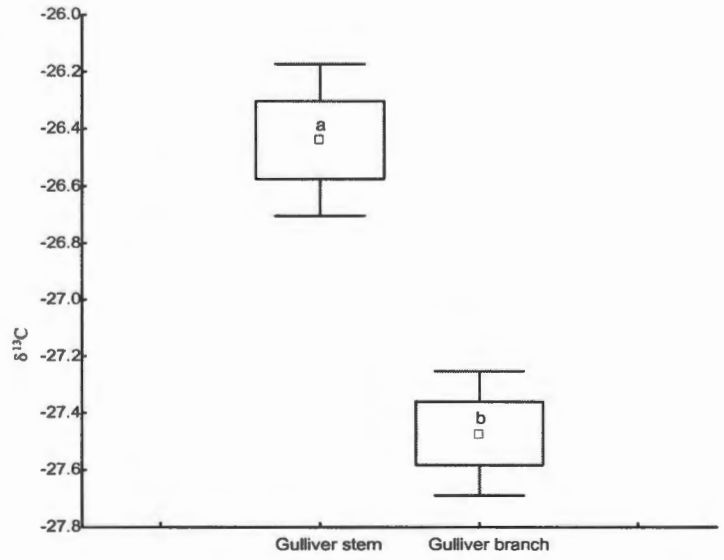

Fig. 11. Mean $\delta^{13}C$ values for the outermost growth of the gulliver stems estimated to cover the same period of growth as the gulliver branches. Differences between a & b were significant (Students t-test; $t = 5.7$, $df = 68$, $p < 0.001$. Means ± SE (box) and 95% confidence intervals (whiskers) are shown.

Discussion

The significantly higher TNC concentrations found in the tubers associated with the "gullivers" may indicate that the gullivers had already completed the "bolting stage" and had sufficient time to replenish their starch reserves. Higher TNC concentrations in the tubers could also have been due to the coppices recharging the tubers prior to the main coppice shoot becoming a gulliver, the growth of which depended on current photosynthate. However, the fast growth rates (2 to 3 m in 3 years) achieved by the gulliver stems probably depends on both carbon reserves and current photosynthate for growth. The extent to which the gulliver relies on stored and current photosynthate is as yet unknown.

The observed TNC concentrations of all tissues measured in this study were relatively high compared to a number of other studies performed on resprouting species. Kays and Canham (1991) measured TNC concentrations in the roots of saplings of four hardwood temperate forest species and found the TNC concentrations to be less than 10% of dry weight. In an attempt to understand how savanna and forest species differ in traits related to fire tolerance, Hoffman *et al.* (2003) compared the TNC concentrations in stems and roots of ten congeneric pairs of species from the Brazilian Cerrado; each pair comprised one forest and one savanna species. No difference in TNC concentration in roots or stems was found between the savanna and forest species. Miyanishi and Kellman (1986) also found relatively low starch concentrations (*ca.* 9 to 14%) in two savanna species. Bond and Maze (unpublished) measured the TNC concentrations of *A. karroo* gulliver tubers every two months over a one year period. They found the TNC concentration to be *ca.* 40% of dry weight. In the current investigation the TNC concentration for *A. karroo* ranged from 24% in coppice tubers to 32% in gulliver tubers. The higher TNC concentrations found in the current investigation and in that of Bond and Maze (unpublished) could possibly indicate a strong dependence of *A. karroo* on stored TNC, possibly due to exposure to frequent fires in the savannas. The species for which TNC concentrations were reported by other authors (Miyanishi and Kellman 1986; Kays and Canham, 1991; Hoffman *et al.* 2003) are likely to employ different strategies for survival in frequently burnt environments (e.g. resistance to fire from thick bark or reproductive maturity within the flame-zone).

Although starch concentrations were higher in the gulliver tubers there was no difference in the size or dry weight of tubers from plants in the two different stages. However, the total available TNC content of the gullivers tubers was higher than that of the coppicing plants. There was no correlation between plant height and tuber dry weight so Gulliver growth was not simply a function of age or size of the root system. However, both TNC concentration and content were correlated with plant height and stem diameter. This correlation could indicate that TNC reserves enabled the extension of the gulliver stem or that bigger plants simply store more TNC. The negative correlation between TNC concentrations and tuber dry weight indicated that smaller tubers had higher TNC concentrations. A possible reason for this is that as the tuber increases in size after each successive fire, more woody material is laid down, thereby decreasing TNC concentrations. The strong correlation between tuber size or dry weight and TNC content indicated that larger tubers were capable of storing more TNC, even though this TNC may have been stored at lower concentrations than in smaller tubers. Thus, as the plants got older the tubers got bigger and contained more carbon reserves. These TNC reserves may enable fast growth rates in the gulliver stage, improving the chance of reaching a height above the flame zone as the plants gets older and tubers get bigger.

One major shortcoming of the use of carbon isotopes to show dependence on heterotrophic growth is that the water use efficiency (WUE) of a plant can also affect the $\delta^{13}C$ signal of plant material. Assimilated carbon becomes more enriched in ^{13}C during times of water stress, while during times of reduced water stress assimilated material becomes depleted of ^{13}C (O'Leary 1980; O'Leary *et al.* 1992; Ehlenringer *et al.* 1993; Rundel *et al.* 1998). The observed pattern in $\delta^{13}C$ across the gulliver and coppice stem cross sections supports the hypothesis that carbon reserves were used for the initial post-burn growth. The $\delta^{13}C$ values in these stages were enriched relative to those of sub-branches from the gulliver canopy and also relative to the $\delta^{13}C$ values of the adults. This enrichment was followed by a gradual depletion in ^{13}C in both the gulliver and coppice stems, indicating a possible switch to increasingly autotrophic growth. It is possible that during this "autotrophic" phase the coppices recharged the tuber TNC content. During this phase, coppice shoot growth could have benefited lack of competition for light as a result of low grass cover after fire and ready access to water as a consequence of the deep roots of *A. karroo* compared to those of the grasses (*pers. observation*).

One of the coppice stems persists to form the "gulliver" stem. This is likely to involve competitive re-allocation of resources from the tuber and possibly also from other coppice stems to the gulliver. Evidence for re-allocation of resources can be derived from the increase in $\delta^{13}C$ which occurs in the gulliver stems while the $\delta^{13}C$ values of the coppice stems continue to decrease across the stem cross-sections (Fig.8A). This is likely to correspond to the period of rapid growth of the gulliver stem, sometimes referred to as the "bolting stage". The $\delta^{13}C$ values thereafter gradually decreased indicating a switch to increasingly autotrophic growth in the gulliver with less and less dependence on stored carbon reserves. During this phase the plants are likely to continue replenishing TNC content of the root tubers. This reserve could serve as "insurance" against the possibility of future fires. This would explain the higher TNC concentrations and content observed in gulliver roots compared to coppice roots.

Because the adults were growing in an area protected from fire (surrounded by large rocks), they are likely to have germinated and grown into adults without having to repeatedly resprout after fires. Thus these adults sampled may not be expected to have the same patterns of $\delta^{13}C$ as observed in the gulliver and coppice stems. The observed patterns of $\delta^{13}C$ in adult stems and sub-branches from the gulliver canopy indicated that $\delta^{13}C$ values of autotrophic plant growth were relatively stable over time. These values seldom varied by more than 1‰ in gulliver canopy branches or over the entire lifespan of the adults. This suggests that seasonal and annual variation in rainfall and temperature at the study site did not cause much variation in the $\delta^{13}C$ values of the plant material. The water use efficiencies of the studied plants therefore cannot be used to explain the observed patterns of $\delta^{13}C$ found in gulliver and coppice stems. The most likely explanation is that initial post-burn regrowth and gulliver growth was based, at least in part, on heterotrophic growth. This is further supported by the fact that the gulliver stems were significantly enriched in $\delta^{13}C$ relative to all coppice and adult organs and gulliver branches taken from the canopy.

Helle and Schleser (2004) found an increase in $\delta^{13}C$ of between 4 and 5‰ in early spring when the growth of temperate forest trees depends on carbon reserves. The $\delta^{13}C$ values then gradually decreased as the plants switched to autotrophic growth, with the lowest $\delta^{13}C$ values found in the late wood of each tree ring. At the very end of each tree ring, the $\delta^{13}C$ values start rising again. This increase was suggested to mark the gradual switchover to storage-dependent growth. Although the differences in $\delta^{13}C$ observed in

present study were not as pronounced as those in the study by Helle and Schleser (2004), the patterns of enrichment and depletion observed were very similar, possibly indicating alternating heterotrophic and autotrophic growth. Since post-fire *A. karroo* has very small above-ground (living) biomass it is possible that autotrophic sources of carbon play a bigger role in these plants than in the trees studied by Helle and Schleser (2004). A further possible reason for the relatively small differences in $\delta^{13}C$ between gullivers and other stages was that in the present investigation, total wood, not extracted cellulose, was used for the isotope analyses. Helle and Schleser (2004) compared the $\delta^{13}C$ values for total wood against extracted cellulose material and found that the total wood material was consistently at least 1‰ more negative than the corresponding extracted cellulose. Therefore, the consistent difference between wood and extracted cellulose is unlikely to explain the smaller variance in $\delta^{13}C$ values found in this investigation.

Conclusions

The lower TNC concentrations found in the coppicing plants indicated the use of TNC reserves for the initial growth of these shoots. This study was unable to determine whether these coppices then recharge the TNC reserves before changing its architecture to a single stem. Although the TNC concentrations were lower in the coppicing plants, they were still relatively high compared to those from other studies performed on both forest and savanna species. The high TNC concentrations and content found in the roots of *A. karroo* probably enables it to survive frequent fires and yet still have the ability to sprout, and repeatedly develop a gulliver, allowing survival and proliferation in areas prone to frequent fires. The isotope analyses support the hypothesis that plants in the gulliver stage utilize stored carbon in order to achieve rapid growth rates.

The lower starch concentrations in the coppicing plants suggest that the plants need more than one year to recharge their reserves. Thus if the plants were burnt annually for an extended period the starch reserves would become depleted, possibly leading to their elimination. This is a possible solution to combating encroaching species; however, the effects of annual burning on other species in these ecosystems would need to be taken into account. Because *A. karroo* plants are so resilient once established, managers could also attempt to prevent further establishment of seedlings by removing reproductive adult plants in areas where *A. karroo* is an encroaching species.

This study showed clear patterns of carbon allocation in post-burn growth of *A. karroo,* however, the dynamics of these patterns appear to be complex. A long-term study with appropriate controls for water use efficiencies would be necessary to gain an understanding of these complex dynamics. Labeled carbon isotopes such as ^{14}C could be used to trace carbon allocation in a plant. This would be the most effective way of determining the allocation of carbon to the different organs of the plant during the different stages in its juvenile life history, and for tracing when stored carbon is remobilized for aboveground growth.

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References

Archer S. 1989. Have southern Texas savannas been converted to woodlands in recent history? American Naturalist 134:545-561.

Balfour D.A. and Howison O.E. 2001. Spatial and temporal variation in a mesic savanna fire regime: responses to variation in annual rainfall. African Journal of Range and Forest Science 19: 43-51.

Bellingham P.J. and Sparrow A.D. 2000. Resprouting as a life history strategy in woody plant communities. Oikos 89: 409-416.

Bell T.L.and Pate J.S. 1996. Growth and fire response of selected Epacridaceae of South Western Australia. Australian Journal of botany 44: 509-526.

Bell T.L., Pate J.S. and Dixon K.W. 1996. Relationships between fire response, morphology, root anatomy and starch distribution in South-West Australian Epacridaceae. Annals of Botany 77: 357-364.

Belsky A.J. 1994. Influences of trees on savanna productivity: Tests of shade, nutrients and tree-grass competition. Ecology 75(4):922-932.

Bond W.J. and Maze K.E. Unpublished. Gullivers: a distinctive life history stage in savanna trees.

Bond W.J. and Midgley J. 2003. The evolutionary ecology of sprouting in woody plants. International Journal of Plant Science 164(3): S103-S114.

Bond W.J. and Midgley G.F. 2000. A proposed CO_2-controlled mechanism of woody plant invasion in grasslands and savannas. Global Change Biology 6: 865-869.

Bond W.J., Midgley G.F. and Woodward F.I. 2003. The importance of low atmospheric CO_2 and fire in promoting the spread of grasslands and savannas. Global Change Biology 9: 973-982.

Bond, W.J. and van Wilgen, B.W. 1996. Fire and plants. Chapman and Hall, London.

Bowen B.J. and Pate J.S. 1993. The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R.Br. (Proteaceae). Annals of Botany 72: 7-16.

Buysse, J. and Merckx, R. 1993. An improved colimetric method to quantify sugar content of plant tissue. Journal of Experimental Botany 44(267):1627- 1629.

Brugnoli E., Hubick K.T., von Caemmerer S., Wong S.C. and Farquhar G.D. 1988. Correlation between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide. Plant Physiology 8: 1418-1424.

Brugnoli E. and Farquhar G.D. 2000 Photosynthetic Fractionation of carbon Isotopes in Leegood R.C., Sharkey T.D. and von Caemmerer (eds), Photosynthesis: Physiology and Metabolism pp. 399-434. Kluwer Academic Publishers, Netherlands.

Chirara C., Frost P.G.H. and Gwarazimba V.E.E. 1998. Grass defoliation affecting survival and growth of seedlings of *Acacia karroo*, an encroaching species in southwestern Zimbabwe. African Journal of Range and Forest Science 15: 41-47.

Duranceau M., Ghashghaie J., Badeck F., Deleens E. and Cornic G. 1999. $\delta^{13}C$ of CO_2 respired in the dark in relation to $\delta^{13}C$ of leaf carbohydrates in *Phaseolus vulgaris* L. under progressive drought. Plant, Cell and Environment 22: 515-523.

Ehlenringer R.E., Hall A.E. and Farquhar G.D. 1993. Stable Isotopes and Plant Carbon-Water Relations. Academic Press, San Diego.

Frost P.G.H., Medina E., Menaut J.C.,Solbrig O., Swift M and Walker B.H. (1986). Responses of savannas to stress and disturbance. Biology International Special Issue 10:1-82.

Gignoux, J., Clobert, J. and Menaut, J.C. 1997. Alternative fire resistence strategies in savanna trees. Oecologia 110:576-583.

Gleixner G., Danier H.J., Werner R.A. and Schmidt H.L. 1993. Correlations between the ^{13}C content of primary and secondary plant products in different cell compartments and that in decomposing Basidiomycetes. Plant Physiology 102: 1287-1290.

Gleixner G., Scrimgeour C., Schmidt H.L and Viola R. 1998. Stable isotope distribution in the major metabolites of source and sink organs of *Solanum tuberosum* L.: a powerful tool in the study of metabolic partitioning in intact plants. Planta 207: 241-245.

Helle G. and Schleser G.H. 2004. Beyond CO_2-fixation by Rubisco – an interpretation of $^{13}C/^{12}C$ variations in tree rings from novel intra-seasonal studies on broad-leaf trees. Plant, Cell and Environment 27: 367-380.

Higgins S.I., Bond W.J. and Trollope W.S.W. 2000. Fire, resprouting and variability: a recipe for grass-tree coexistence in savanna. Journal of Ecology 88: 213-229.

Hobbie E.A. and Werner R.A. 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C_4 plants: a review and synthesis. New Phytologist 161: 371-385.

Hodgkinson K.C. 1998. Sprouting success of shrubs after fire: height-dependent relationships for different strategies. Oecologia 115: 64-72.

Hoffmann T., Todd, S., Ntshona Z. and Turner S. (1999). Land degradation in South Africa. Department of Environmental Affairs and Tourism, Pretoria. pp 126-134.

Hoffman W.A., Bazzaz F.A., Chatterton N.J., Harrison P.A. and Jackson R.B. 2000. Elevated CO_2 enhances resprouting of a tropical savanna tree. Oecologia 123: 312-317.

Hoffman W.A., Orthen B. and Nascimento P.K.V.D. 2003. Comparitive fire ecology of tropical savanna and forest trees. Functional Ecology 17: 720-726.

Jaggi M., Saurer M., Fuhrer J. and Siegwolf R. 2002. The relationship between the stable carbon isotope composition of needle bulk material, starch, and tree rings in *Picea abies*. Oecologia 131: 325-332.

Kays J.S. and Canham C.D. 1991. Effects of time and frequency of cutting on hardwood root reserves and sprout growth. Forest Science 37(2): 524-539.

Le-Roux-Swarthout D.J., Terwilliger V.J. and Martin C.E. 2001. Deviation between $\delta^{13}C$ and leaf intercellular CO_2 in *Salix interior* cuttings developing under low light. International Journal of Plant Science 162(5): 1017-1024.

Matheson W. and Ringrose S. 1994. Assessment of degradation features and their development into the post-drought period in the west-central Sahel using Landsat MSS. Journal of Arid environments 15:12-26.

Maze K.E. 2001. Fire survival and life histories of Acacia and Dichrostachys species in a South African Savanna. Unpublished Master's thesis. University of Cape Town.

Miyanishi K. and Kellman M. 1986. The role of root nutrient reserves in regrowth of two savanna shrubs. Canadian Journal of Botany 64: 1244-1248.

Moleele N.M., Ringrose S., Matheson W. and Vanderpost C. 2002. More woody plants? The status of bush encroachment in Botswana's grazing areas. Journal of Environmental Management 64: 3-11.

O'Connor T.G. 1995. *Acacia karroo* invasions of grassland: environmental and biotic effects influencing seedling emergence and establishment. Oecologia 103: 214-223.

O'Leary M.H. 1981. Carbon istope fractionation in plants. Phytochemistry 20(4): 553-567.

O'Leary M.H., Madhaven S. and Paneth P. 1992. Physical and chemical basis of carbon isotope fractionation in plants. Plant, Cell and Environment 15: 1099-1104.

Rundel P.W., Ehleringer J.R. and Nagy K.A. (editors). 1989. Stable Isotopes in Ecological Research. Springer-Verlag, New York.

Scholes, R.J. and Archer, S.R. 1997. Tree-grass interactions in savannas. Annual Review of Ecology and Systematics 28:517-544.

Skowno A.L., Midgley J.J., Bond W.J. and Balfour D. 1999. Secondary succession in *Acacia nilotica* (L.) savanna in the Hluhluwe Game Reserve, South Africa. Plant Ecology 145: 1-9.

Terwilliger V.J. and Huang J. 1996. Heterotrophic whole plant tissues show more ^{13}C enrichment than their carbon sources. Phytochemistry 43(6): 1183-1188.

Trollope W.S.W. 1987. Effect of season of burning on grass recovery in the false thornveld of the Eastern Cape. Journal of the Grasslands Society of South Africa 4(2):74-77.

Wand S.J., Midgley G.F. and Musil C.F. 1996. Physiological and growth responses of two African species, *Acacia karroo* and *Themeda triandra*, to combined increases in CO_2 and UV-B radiation. Physiologia Plantarum 98: 882-890.

Whately A. and Porter R.N. 1983. The woody vegetation communities of the Hluhluwe-Corridor_Umfolozi Game Reserve Complex. Bothalia 14(3&4):745-758.

Williams R.J., Duff G.A., Bowman D.M.J.S. and Cook G.D. 1996. Variation in the composition and structure of tropical savannas as a function of rainfall and soil texture along a large scale climatic gradient in the Northern Territory, Australian Journal of Biogeography 23:747-756.