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The evaluation of $\delta^{13}C$ isotopes of trees to determine past regeneration environments

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Abstract

The effects of past regeneration environments and canopy position on $\delta^{13}C$ signals in leaf and wood tissue were examined. Leaves were collected from various canopy positions both inside and outside of closed forest and from gaps ranging in size from 75 to 829 m^2. Trees of known recruitment environments were cored and wood was extracted from the outer rings and from the centre of the tree. Whole tissue was converted to holocellulose for isotopic analysis. An elevation of ~1‰ in $\delta^{13}C$ was associated with a conversion from whole wood to holocellulose. A regression of whole tissue vs. holocellulose produced an R^2=0.84. A significant depression in $\delta^{13}C$ values of leaf tissue was observed in areas under a closed canopy. Leaves sampled from open areas, or from a well-lit canopy position, had more positive $\delta^{13}C$ values. In gaps, $\delta^{13}C$ of the leaves increased with increasing gap size. The existence of a significant difference between $\delta^{13}C$ of inner and outer woods within a tree indicates that it is possible to distinguish between differing recruitment environments with this technique. The data indicate that the regeneration environment confers a specific isotopic signal on a tree that can be detected later in its life-span. The method can be used to determine recruitment environments and should allow for grouping of species into functional types. The potential uses of this ecological tool are important for restoration ecology and the management of forest ecosystems.

Keywords: Canopy position; Forest ecology; Functional types; Gaps; Stable carbon isotopes; Tree cores

1. Introduction

In tropical and sub-tropical forests, regeneration requirements of trees are central to understand forest dynamics. Trees vary in their ability to establish in shade or in gaps of different sizes. Identification of gap requirements is not always possible from analysing the distribution of young trees. This is the case in southern African sub-tropical forests, where one often sees a lack of regeneration of canopy trees (Midgley et al., 1995b). In this study, we explore the use of stable carbon isotopes as a tool to determine the regeneration history of forest trees and its application in interpreting patch-scale dynamics in sub-tropical forests.

The $^{13}C/^{12}C$ ratio of plant tissue varies according to a variety of factors including carbon source, CO_2 concentration, O_2 concentration, light intensity and water use efficiency (Smith et al., 1976; Ehleringer et al., 1986; Farquhar et al., 1989; van der Merwe and Medina, 1989; Leavitt and Long, 1991; Buchmann et al., 1997; Hanba et al., 1997). In forest ecosystems, three main factors governing variation in $\delta^{13}C$ are carbon source, CO_2 partial pressures internal and

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external to the leaf, and water-use efficiency (Berry et al., 1997). As carbon is cycled under the canopy, it becomes isotopically lighter (depleted in ^{13}C) than normal atmospheric CO_2 (Vogel, 1978). Plants that photosynthesise below the canopy and utilise this sub-canopy CO_2 become isotopically light. Plants with their leaves in or above the canopy draw on CO_2 from the atmospheric pool, which is richer in ^{13}C, and thus differ from sub-canopy plants.

The ratio of the partial pressures of CO_2 internal and CO_2 atmospheric (c_i/c_a) is also responsible for the isotopic differences in foliage seen in forest communities. These differences can be strongly correlated to light intensity (Leavitt and Long, 1991). As light intensity drops, c_i/c_a increases leading to a discrimination against the heavier isotope and an isotopically lighter plant. Water-use efficiency also contributes to c_i/c_a and works in a similar direction to that of light intensity. As water stress increases, stomatal conductance decreases enhancing water-use efficiency (Farquhar et al., 1989; Schulz and Adams, 1995). This causes a decrease in c_i/c_a which favours the heavier isotope and leads to an increased (less negative) $\delta^{13}C$ signal (Leavitt and Long, 1991). Thus, in a forest community, one can envision trees in well-lit open areas being isotopically heavier than shaded, sub-canopy trees due to the combined effects of carbon source, light intensity and water-use efficiency.

These factors lead to the pattern seen in forests of decreasing $\delta^{13}C$ down the canopy profile (Vogel, 1978; Medina and Minchen, 1980; Ehleringer et al., 1986; van der Merwe and Medina, 1989; Medina et al., 1991). This effect has been shown with regard to canopy structure (Buchmann et al., 1997), irradiance clines (Ehleringer et al., 1986; Hanba et al., 1997) and to a limited extent in cleared forest areas (van der Merwe and Medina, 1989).

In our study, three lines of evidence were examined. Firstly, $\delta^{13}C$ signals from shaded and well-lit leaf tissue were examined. This tested for expected differences between $\delta^{13}C$ signals in leaves, in shaded or open positions during different stages in the life history of a tree. Secondly, the effects of gap size on $\delta^{13}C$ variation in leaf tissue were examined. Thirdly, the $\delta^{13}C$ signals of early and late woods in tree cores were examined in trees of various regeneration locations (shaded and open) to test for changing and growing conditions during the life of a tree.

2. Materials and methods

2.1. Sites

Sampling was undertaken in mature forest and neighbouring open areas at Diepwalle Forest Reserve in Knysna and Hilltop Forest in Hluhluwe Game Reserve, South Africa (Fig. 1). The study sites represent two very different forest types in South Africa, Diepwalle being temperate and Hilltop sub-tropical forest (Table 1). These forests have also been labelled fine-grained and coarse-grained, respectively (Midgley et al., 1990), with reference to their regeneration patterns, characterised by shade-tolerant and shade-intolerant species, respectively. For this study, six species were sampled: *Podocarpus latifolius*, *Afrocarpus falcatus* and *Olea capensis subsp. macrocarpa* (Diepwalle), *Protorhus longifolia*, *Chrysophyllum viridifolium* and *Celtis africana* (Hilltop). *P. latifolius*, *O. capensis macrocarpa* (Midgley et al., 1990) and *C. viridifolium* (West, unpublished data) are described as shade-tolerant. *C. africana* is a facultative species (Everard et al., 1995). *A. falcatus* (Midgley et al., 1990) and *P. longifolia* (West, unpublished data) are described as shade-intolerant.

Table 1
Site characteristics and species sampled

Characteristics	Forest type/site name	
	Temperate/Diepwalle forest	Sub-tropical/Hilltop forest
Latitude	33°58′S	28°00′S
Longitude	23°05′E	31°43′E
Elevation	519 m a.s.l.	750 m a.s.l.
Precipitation p.a.	1187 mm[a]	990 mm[b]
Canopy height	20–30 m	12–20 m
Species sampled	*P. latifolius*	*P. longifolia*
	A. falcatus	*C. africana*
	O. capensis macrocarpa	*C. viridifolium*

[a] Source: Midgley et al. (1990).
[b] Source: Brooks and Macdonald (1983).

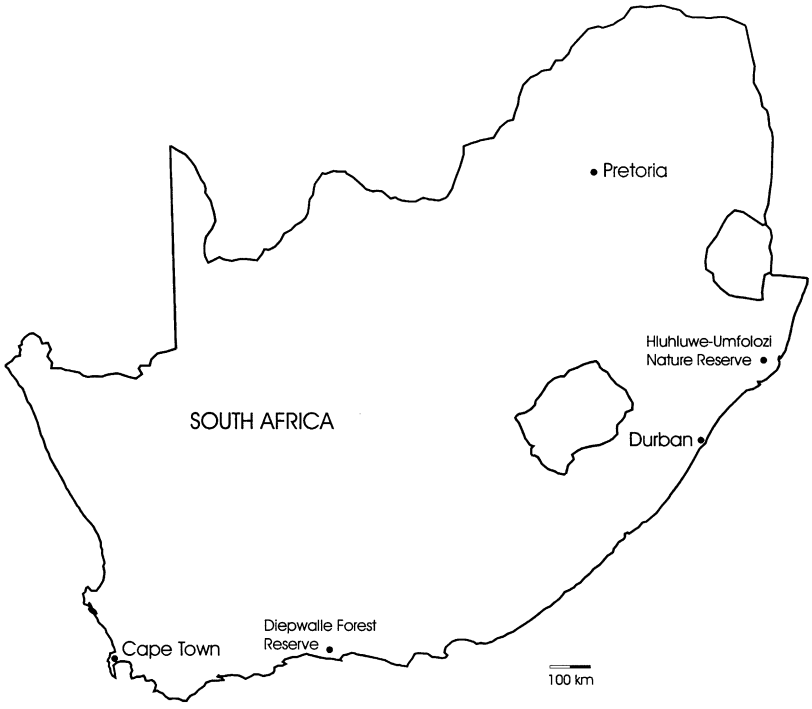

Fig. 1. Map of the South Africa showing the position of the Hluhluwe–Umfolozi Game Reserve and the Diepwalle Forest Reserve.

2.2. Sample collection

Sampling took the form of leaf collection and tree coring during the period from March to July 1998.

2.2.1. Leaf samples

Leaves were collected from fully shaded juveniles and from the top of emergent canopy trees in the closed canopy forest. Comparable sampling was performed, outside of the forest, in open areas. Here the juveniles were fully lit, as were the mature trees. In all the cases, five trees were sampled from each category with *circa* 20 fully expanded new leaves being collected from all four cardinal points of the individual.

2.2.2. Leaf samples — gaps

Thirty-one gaps of various sizes (75–829 m^2) were sampled for juvenile sun leaves. The gaps sampled were part of an experiment by the Department of Forestry. These gaps were cleared in the Diepwalle forest and in the Tsitsikamma Nature Reserve in 1994, and their exact size, position and contents were noted. It was thus possible to sample, with confidence, individuals that established after the creation of the gap.

2.2.3. Tree cores

We first tested the values of $\delta^{13}C$ for identifying recruitment conditions by coring trees of known history. We then applied this method to trees of unknown regeneration history. For trees of known history, one group encompassed trees that recruited and grew up completely in the open, never having been shaded for any length of time. This group is hereafter referred to as ''open''. Trees for this category were selected from an arboretum (Temperate forest) where trees were planted in cleared forest areas.

The section of the arboretum used in this study was created in 1931, when 1.23 ha of forest was cleared and monospecific stands of forest trees were planted in

0.28 ha (2800 m^2) areas (Lübbe and Geldenhuys, 1991). These areas were repeatedly cleared of all other plants until 1969 (Lübbe and Geldenhuys, 1991). The arboretum enabled the collection of genuine ''open'' samples of shade-tolerant species, effectively an artificial situation, which could then be contrasted against the natural scenario of recruitment under a closed canopy.

In the sub-tropical forest, ''open'' samples were obtained from expanding forest margins. The ''open'' trees were compared with trees that recruited and grew up in the shade and subsequently emerged though the canopy to be fully lit. This category is hereafter referred to as ''closed''. As detailed records of individual trees in these forests are not available, trees were selected by choosing an individual presently in the canopy that was surrounded by larger individuals on all sides. It was then inferred that these older trees would have shaded the selected tree until it eventually reached the canopy. This assumption is valid for the shade-tolerant species. However, for shade-intolerant species, caution must be exercised when interpreting such ''closed'' situations as it is unlikely that these species would establish and grow in the shade. Nevertheless, for the sake of consistency, these cores were labelled as ''closed''.

The method was then applied to trees of unknown regeneration history. These trees were suspected of recruiting in abandoned agricultural fields within the sub-tropical forest. They formed an apparent cohort of similar sized stems grouped on roughly a hectare of level ground within a much larger forest. Isotopic analysis of the soil carbon in these areas showed the existence of C_4 plants at some stage in the past. This group is hereafter referred to as ''field''.

2.2.4. Sampling tree cores

Tree coring was performed with borers of two different sizes. The larger borer (24 mm diameter) was used to remove the first 2 cm of wood from the tree, the bark and phloem being discarded. The borer was then drilled in further to stop 1.5 cm short of the estimated centre of the tree. The large borer was then removed and a stainless steel pipe (20 mm diameter) was inserted and held against the drilling face. Into this pipe, the small borer (19 mm diameter) was inserted, and 3 cm of wood removed. The wood was then pulled out through the pipe thereby preventing contamination with wood along the core. Wounds were then filled with dowel and sealed. Cores were taken on the trunk as low as possible.

Care was taken to estimate the centre of the tree accurately, but it can be assumed that this was not always achieved hence the larger section of wood being taken from the centre of the tree in order to maximise the chance of striking the centre. This is an inherent weakness of the method but unfortunately seems unavoidable if a principle of non-destructive sampling must be adhered to, as was the case in this study.

2.3. Sample analysis

Samples were oven dried at 70°C for 48 h and then ground in an electric mill to fine powder. Cellulose (holocellulose) was isolated from the samples using a toluene–ethanol solvent and acidified sodium chlorite solution (Leavitt and Danzer, 1993). A 0.05 mg subsample of this holocellulose was then combusted on-line in a VG Micromass 602E mass spectrometer and the isotopic ratio of the CO_2 was determined relative to the PDB standard [$\delta^{13}C=((R_{sample}/R_{PDB})-1\times1000)$, where R is the ratio of ^{13}C to ^{12}C].

2.4. Data analyses and statistics

For leaf samples, differences between categories were tested by ANOVA. Homogeneity of variance was tested with a univariate test (Cochran, Hartley and Barlett) and ANOVA only run if no significant difference was obtained. Means were tested with the Tukey Honest significant difference test.

ANOVAs were also used to test for a significant difference between $\delta^{13}C$ signals from small (<400 m^2) and large (>400 m^2) gaps. Comparisons of $\delta^{13}C$ signals from closed forest, small gaps, large gaps and open areas were performed by ANOVA, where the variance was homogeneous. Where variance was not homogeneous (*O. capensis macrocarpa* and *P. latifolius*), the Kruskal–Wallace test was employed.

The means and standard deviation of the difference between $\delta^{13}C$ of inner and outer wood within a tree core were calculated and plotted. ANOVAs were performed between ''open'' and ''closed'' cores within the

same species. ANOVAs were also performed between "open" and "closed" cores within shade-tolerant and shade-intolerant categories. These categories were based on previous knowledge of these species' traits as mentioned earlier.

3. Results

3.1. Holocellulose vs. whole tissue

One hundred and twenty-five samples were run both as whole tissue and as holocellulose. Eighty-one of these samples were derived from leaf tissue and 44 from core wood. The overall regression of holocellulose plotted against whole tissue (Fig. 2) produced an R^2=0.84. For leaf tissue and core wood, the R^2 was 0.74 and 0.84, respectively. For each case, the regression line was elevated by approximately 1‰ from a one to one relationship. The residuals of each group of samples were examined for specific trends. There was no apparent trend of consistent elevation or depression away from the regression amongst any of the categories sampled.

3.2. Carbon isotope ratios of foliage

Within a species, sun leaves from canopy trees in closed forest, leaves from similar sized trees and juvenile trees located outside the forest had similar $\delta^{13}C$ values (Fig. 3). In all species, leaves from juvenile trees located under a closed forest canopy were consistently and significantly more negative than all other cases (Fig. 3). An increase in $\delta^{13}C$ is seen with increasing gap size (Fig. 4). This increase is linear; however, one would expect an asymptotic relationship with $\delta^{13}C$ tending to a maximum at the larger gap sizes.

We determined that 400 m^2 was an appropriate area above and below which it was possible to distinguish between regeneration in large and small gaps. The difference between $\delta^{13}C$ values above and below 400 m^2 was significant for all the three species (*P. latifolius*: $F_{(1,\ 13)}$=17.67, p<0.0010; *O. capensis*

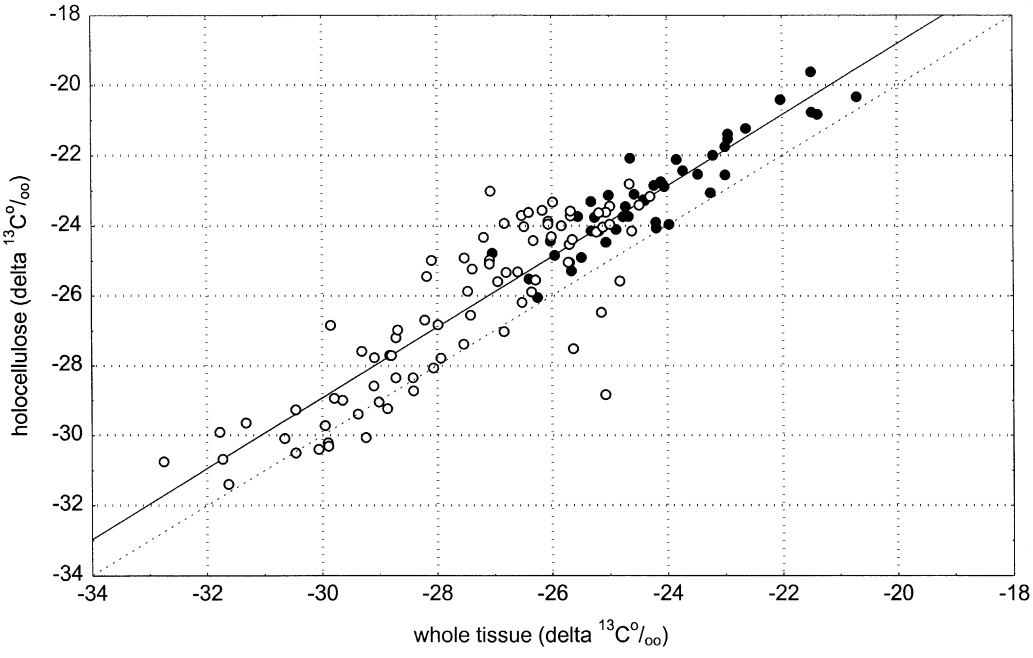

Fig. 2. Graph showing the regression of $\delta^{13}C$ of whole tissue vs. $\delta^{13}C$ of holocellulose for leaf (open circles) and core (filled circles) samples (R^2=0.84, n=125). The solid line indicates the regression. The dashed line shows a hypothetical one-to-one relationship between whole tissue and holocellulose.

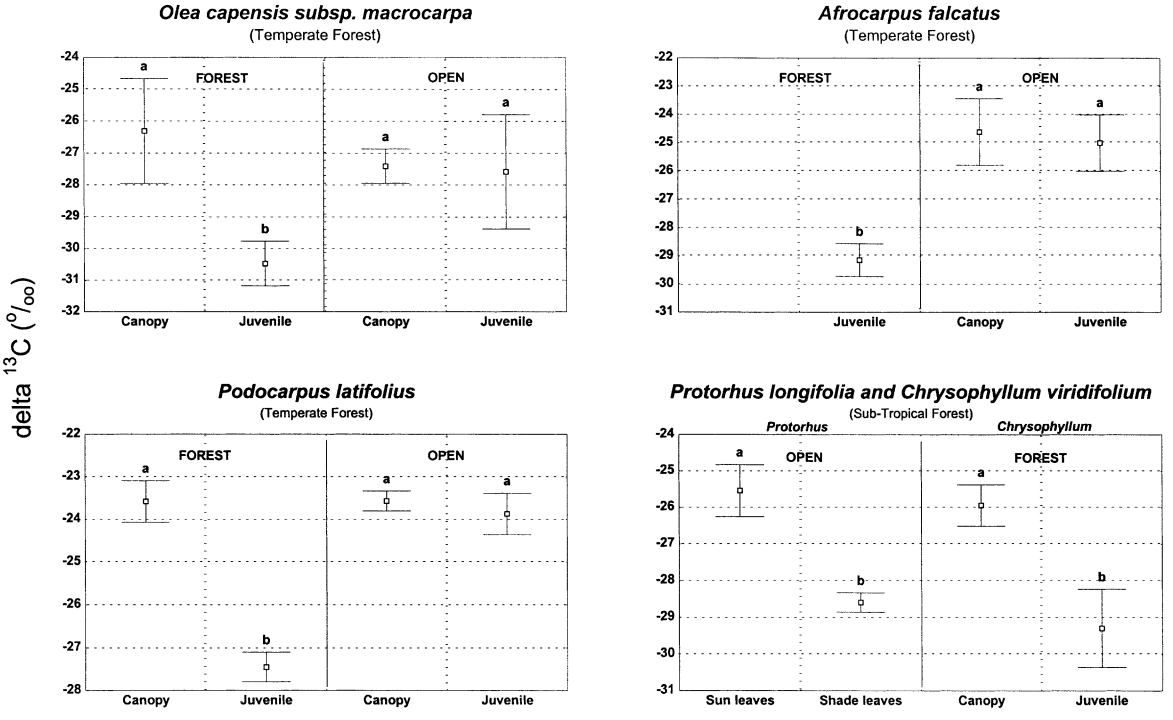

Fig. 3. $\delta^{13}C$ values for holocellulose extracted from leaf tissue showing the effect of habitat and canopy position in temperate and sub-tropical forests. "Forest" and "open" indicate habitat. Where not specified, all "open" and "forest" canopy leaves were fully lit. All "forest" juveniles were fully shaded. Letters indicate significantly different means (Tukey HSD test).

macrocarpa: $F_{(1,\ 10)}=24.52$, $p<0.0006$; *A. falcatus*: $F_{(1,\ 6)}=6.58$, $p<0.0426$). There is a trend of increasing $\delta^{13}C$ with increasing area of exposure to both light and atmospheric CO_2 (Fig. 5). Large and small gaps generally induce a $\delta^{13}C$ closer to the open and closed environments, respectively, than to each other (Fig. 5).

3.3. Carbon isotope ratios of tree cores

We used the difference in $\delta^{13}C$ between inner and outer woods as a measure of ontogenetic change in growing conditions. Since shaded leaves have more negative $\delta^{13}C$ than exposed canopy leaves, plants that regenerate in the shade should show a more negative difference between inner and outer woods than plants that established in open or large gap environments.

The means of the differences between the $\delta^{13}C$ of inner and outer woods are shown in Figs. 6 and 7. For the shade-tolerant species (Fig. 6), namely, *P. latifolius*, *O. capensis macrocarpa*, *C. africana* and *C. viridifolium*, there is a relatively clear distinction between "closed" trees, which have a negative differential, and "open" and "field" trees, which have a positive differential.

For the shade-intolerant species, *A. falcatus* and *P. longifolia*, all means are positive for "closed", "open" and "field" categories (Fig. 7).

There was a significant difference between "open" and "closed" core categories for the shade-tolerant species *P. latifolius* ($F_{(1,\ 8)}=22.46$; $p<0.0015$) and *O. capensis macrocarpa* ($F_{(1,\ 8)}=10.67$; $p<0.0114$). There was no significant difference between the "open" and "closed" categories for the shade-intolerant species *A. falcatus* ($F_{(1,\ 8)}=0.56$; $p<0.4738$) and *P. longifolia* ($F_{(1,\ 8)}=0.20$; $p<0.6659$).

"Open" and "closed" categories were compared against each other for both the shade-tolerant (Fig. 6) and shade-intolerant species (Fig. 7). A significant difference was seen between "open" and "closed" for

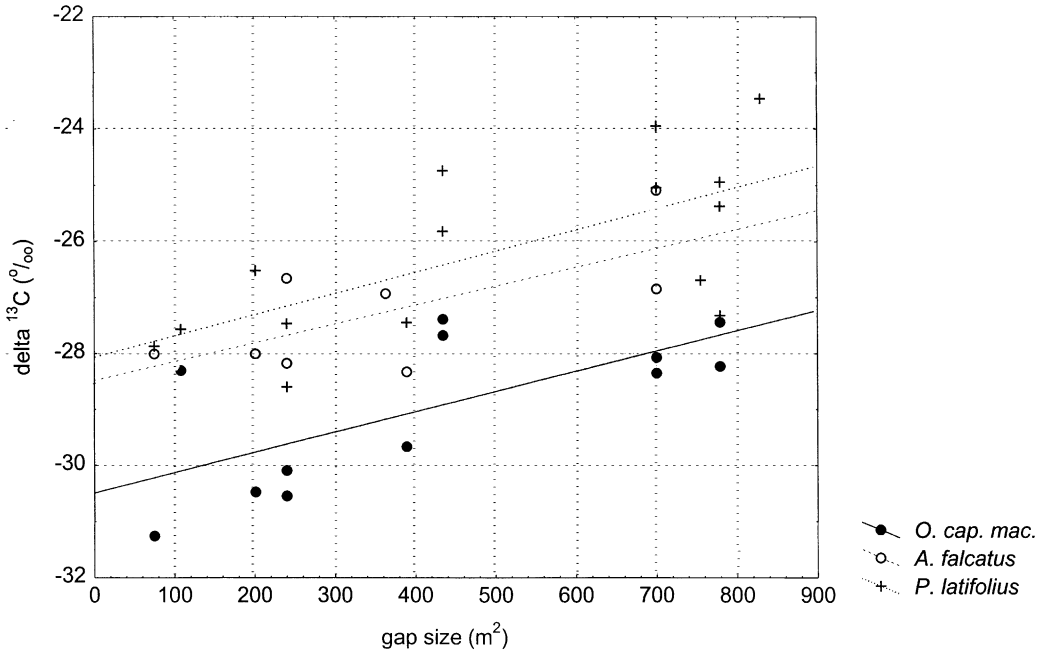

Fig. 4. $\delta^{13}C$ of leaf holocellulose vs. gap area, in a temperate forest, for seedlings of *O. capensis macrocarpa* (R^2=0.47), *P. latifolius* (R^2=0.46) and *A. falcatus* (R^2=0.48). Individuals sampled were well-lit and located in the centre of the gap. All individuals were of similar size, between 30 cm and 1 m in height.

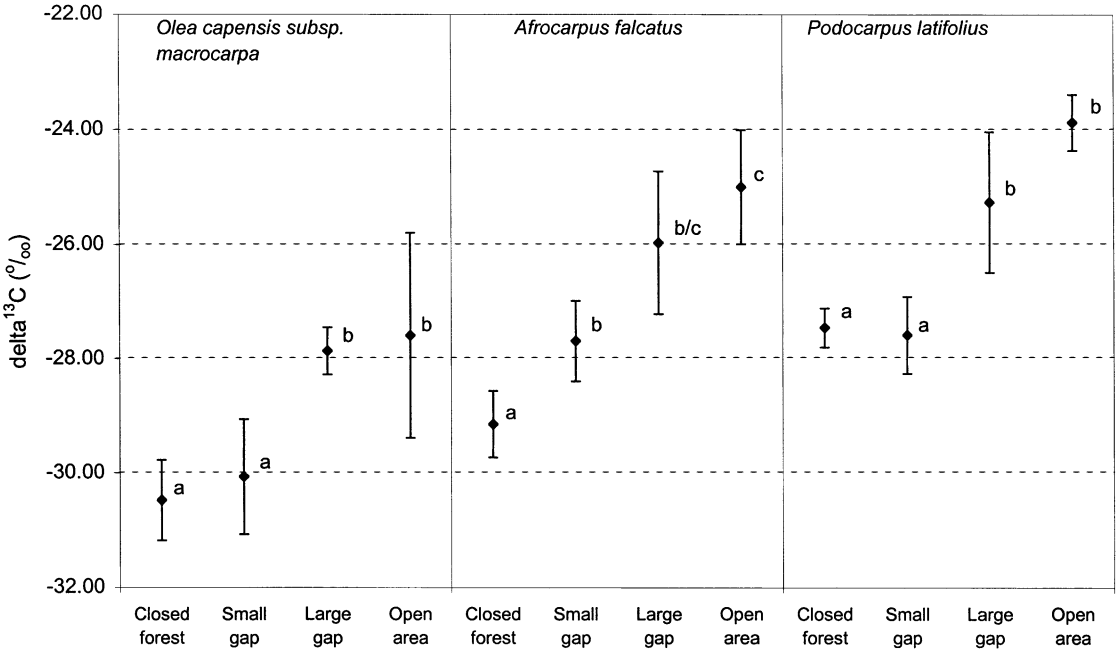

Fig. 5. Comparison of $\delta^{13}C$ of holocellulose from juvenile leaves from closed forest, small gaps (<400 m^2), large gaps (>400 m^2) and open areas. Within a species, different letters indicate significantly different means.

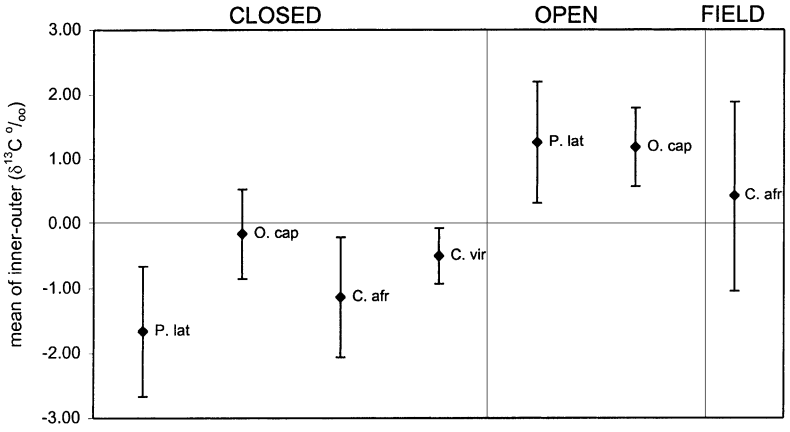

Fig. 6. Means of the differences between $\delta^{13}C$ of inner and outer wood for shade-tolerant canopy tree species *P. latifolius*, *O. capensis macrocarpa*, *C. africana* and *C. viridifolium*. Groups on the *x*-axis refer to the recruitment environment of the tree. "Closed" indicates recruitment beneath a canopy, "open" indicates recruitment in open areas and "field" indicates recruitment in abandoned fields.

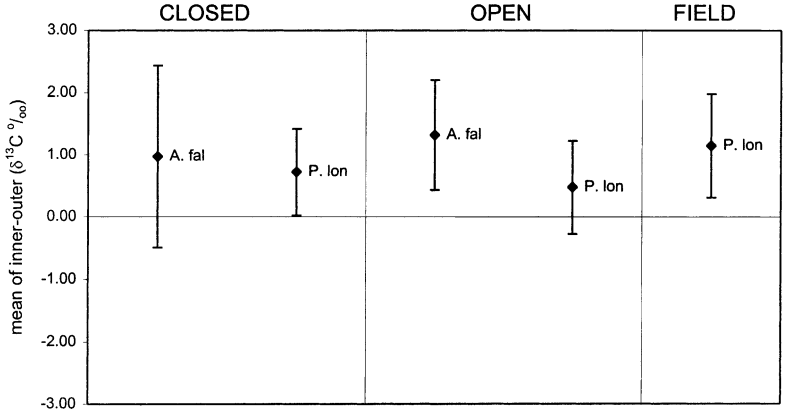

Fig. 7. Means of the differences between $\delta^{13}C$ of inner and outer wood for shade-intolerant canopy tree species *A. falcatus* and *P. longifolia*. Groups on the *x*-axis refer to the recruitment environment of the tree. "Closed" indicates recruitment beneath a canopy, "open" indicates recruitment in open areas and "field" indicates recruitment in abandoned fields.

the shade-tolerant species ($F_{(1, 26)}=35.37$; $p<0.0000$), but not for the shade-intolerant species ($F_{(1, 18)}=0.02$; $p<0.8927$).

4. Discussion

Our results indicate that it is possible to determine past regeneration environments of mature trees by using a simple isotopic test. The method is based upon the fractionation of $\delta^{13}C$ due to the changes in source CO_2, light intensity and water-use efficiency.

4.1. Cellulose vs. whole tissue for isotopic study

This study examines small changes in $\delta^{13}C$ during the individual's life-span, a signal that is contained in the cellulose laid down at that time. Whole tissue contains mobile compounds, such as lipids, starch and sugars, that have characteristically different $\delta^{13}C$

signals (Benner et al., 1987; Boutton, 1996) and can thus obscure the cellulose signal. We were interested to know if, practically, extraction of cellulose (holocellulose) was a necessary procedure for this study. The regressions of whole tissue to holocellulose (Fig. 2) showed a good fit, and a positive shift of 1‰ for whole tissue would calibrate it to the holocellulose regression. However, there is no pattern in the dispersion of residuals away from the regression and this prohibits the use of whole tissue $\delta^{13}C$ especially if sample size per category is low, as was the case for this study. We concluded that extraction of holocellulose from whole tissue is required for accurate results in studies with low sample size examining small $\delta^{13}C$ shifts.

4.2. Testing the hypothesis: leaf tissue

The study on leaf tissue served to test for the effects of the factors outlined above on $\delta^{13}C$ signals. The results supported the a priori hypothesis that the combined effects of shading, water-use efficiency and CO_2 recycling under a canopy should produce a more negative isotopic signal. All ''closed'' juveniles had a significantly more negative $\delta^{13}C$ signal than the juveniles in gaps or in open areas (Figs. 4 and 6). This was the case in both temperate and sub-tropical forests.

The results also showed a distinct difference between leaf isotope composition in small gaps (<400 m^2) and large gaps (>400 m^2) (Fig. 4). Small gaps and large gaps were found to be more similar to closed forest and open areas, respectively, than to each other (Fig. 5), a result that concurs with work done in Puerto Rico (Medina et al., 1991). These results indicate a gradient of increasing $\delta^{13}C$ with increasing gap size. More importantly, they indicate the relative lack of importance of small gaps in affecting the $\delta^{13}C$ signal of the vegetation. Average gap sizes reported in other forests are generally below 400 m^2 (Midgley et al., 1995a; Kneeshaw and Bergeron, 1998). In Hilltop forest, of 20 gaps sampled, average gap size was 265 m^2 (West, unpublished data). Thus, it could be that most treefall-generated forest gaps are relatively inconsequential in terms of significantly influencing the $\delta^{13}C$ of the plant. This has important consequences for the interpretation of $\delta^{13}C$ signals in tree cores. Trees that recruit and grow up through a canopy should maintain a low $\delta^{13}C$ regardless of whether they are periodically lit by the small gaps that often puncture a continuous canopy. Thus, any positive deviation of $\delta^{13}C$ within a tree core cannot be linked to release via small gaps in a closed forest community.

We deduce, from the leaf data, that the analysis of tree cores is feasible and should contain meaningful information regarding past regeneration environments.

4.3. Tree cores as records of past regeneration environments

Despite the inherent difficulties of sampling and interpreting $\delta^{13}C$ signals of tree cores, it is possible to derive useful ecological data from them. Using the method developed in this study, it is possible to distinguish between different life-histories of individual trees and, in doing so, to determine their past regeneration environments and hence their current regeneration requirements.

Tree cores were analysed by subtracting the $\delta^{13}C$ value of the most recent (outer) wood from that of the oldest (inner) wood. This creates a relative measure, or differential, per tree that can then be compared with other individuals. We discovered that trees that had recruited in the shade, beneath a closed canopy, and then subsequently reached the canopy (''closed'') displayed a negative differential (Fig. 6). Trees that recruited in open environments that were separate from the forest (''open'') displayed a positive differential (Figs. 6 and 7).

At first sight, the values for ''closed'' shade-intolerant trees (Fig. 7) seem to be an exception to the rule. These trees were categorised as ''closed'' because they were smaller canopy individuals surrounded by larger canopy individuals. However, being shade-intolerant, they are incapable of recruiting and actively growing in the shade. These are therefore examples of trees that recruited in similar situations to their ''open'' neighbours, but subsequently have been out-competed and over-topped. This history is known to be the case for *A. falcatus* in the Diepwalle arboretum. We regard the fact that these ''closed'' trees return the same differential as their ''open'' counterparts as a vindication of the method.

The negative differential seen in trees recruiting below the canopy (Fig. 6) conforms to the patterns

seen in the leaf data. The inner wood has a more negative $\delta^{13}C$ signal as it is a product of sub-canopy photosynthesis. The outer wood is primarily a product of full sun photosynthesis by canopy leaves, hence the more positive signal.

The positive differential of the ''open'' trees denotes that the outer wood had a more negative signal than the outer wood. As the source CO_2 is constant in this case, this indicates that the tree experiences a greater degree of shading and/or decreased water-use efficiency with age. Both of these factors are possible, as self-shading of leaves and reduced water stress are likely to occur in mature trees. In fact, seedlings recruiting in open environments are likely to be highly water stressed and experience almost no shading, hence the more positive $\delta^{13}C$ signal in the inner wood.

4.4. Application: regeneration in a southern African sub-tropical forest

To determine the utility of the method, we investigated the regeneration habitats of canopy dominants in Hilltop Forest, Hluhluwe Game Reserve. Sub-canopy recruitment and gap replacement do not presently occur in these forests (West, unpublished data). The exact mechanism of regeneration is unknown, however, large-scale disturbance is suspected to trigger recruitment in these forests.

An analysis of tree cores from patches within the forest showed a prevalence of positive differentials (''Field'' cores, Figs. 6 and 7), suggesting recruitment in open areas or large gaps. This result suggests that large-scale disturbance is in fact an important regeneration mechanism in this forest.

Thus, the isotopic test for determining past regeneration environments developed in this study is effective. It is possible to distinguish between shade-tolerant and shade-intolerant species as well as the life-histories and past regeneration environments of individual trees. More precise sampling of the tree cores would enhance the accuracy of this method.

Applications for this method are primarily in forest dynamics and restoration ecology. The method is useful for reconstructing past environments and determining regeneration requirements of species and could be applied anywhere where stochastic or infrequent regeneration occurs. It can also be used as a technique to determine degree of shade-tolerance of individual trees and, from this, to classify species into functional types. Possibly, this method could be extended and used to distinguish between sprouting and seeding forest trees.

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