

# Soil carbon isotopes reveal ancient grassland under forest in Hluhluwe, KwaZulu-Natal

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**We investigated stable carbon isotope ratios of soil organic matter in the forests of the Hluhluwe-Umfolozi Game Reserve, KwaZulu-Natal. Analysis of soil profiles in the forest indicated a shift from C<sub>3</sub> to C<sub>4</sub> vegetation with depth. These results suggest that the area now covered by mature, tall forest in the region was once grassland. These findings support the hypothesis that the grasslands of KwaZulu-Natal are older than previously thought.**

The origin and spread of grassland vegetation in South Africa has intrigued biologists. Grasslands have been viewed as being of recent origin, spreading at the expense of forests under the influence of anthropogenic burning, farming, felling and pastoralism.<sup>1,2</sup> The age of grasslands elsewhere has recently been revised, however, placing their origin in the late Miocene and spreading to cover large areas by the Pleistocene, long before human impact was of any significance.<sup>3–5</sup> Authors in South Africa have supported this concept of ancient grasslands,<sup>6,7</sup> challenging the view that Iron Age farmers were responsible for the extent of grasslands in South Africa. Instead, it seems that farmers had only localized effects on forest/grassland boundaries and that the extent of grasslands owes more to biophysical processes operating on large temporal and spatial scales.

In this paper we report the results of an analysis of isotopic composition of soil organic matter (SOM) in the Hluhluwe Forest. The forest forms an 'island' in an area thought to have been deforested by Iron Age farmers, leading to the surrounding savanna structure of today.<sup>2</sup> Our results indicate that the Hluhluwe Forest, far from being an ancient relic of a forested landscape, was previously a grassland. Although we have not obtained dates in this study, our preliminary results are consistent with evidence from elsewhere in Africa for a general increase in tree and forest cover since the start of the Holocene.<sup>8,9</sup>

## Soil organic matter carbon isotopic studies

C<sub>3</sub> and C<sub>4</sub> plants have different <sup>13</sup>C/<sup>12</sup>C ratios and produce very different isotopic signatures.<sup>10</sup> The mean δ<sup>13</sup>C value for C<sub>3</sub> plants is –27‰ and for C<sub>4</sub> plants is –13‰, with considerable variation about the mean.<sup>11,12</sup> The δ<sup>13</sup>C signal of the vegetation is conveyed to the SOM with decay<sup>13–16</sup> and may persist for from decades to millennia.<sup>4,17</sup> Under stable conditions the δ<sup>13</sup>C signature of the surface SOM is in equilibrium with the vegetation from which it is derived.<sup>18</sup> Older SOM, lower down the soil profile, should also reflect the δ<sup>13</sup>C signature of the parent vegetation.<sup>19</sup> Thus, under conditions of vegetation change, the δ<sup>13</sup>C value of the soil profile should offer a record of the dominant vegetation type through time. However, progressive enrichment of δ<sup>13</sup>C of SOM occurs<sup>20,21</sup> This is attributed to recent changes in the <sup>13</sup>C content of atmospheric CO<sub>2</sub>,<sup>22,23</sup> the differential preservation of SOM

components with different isotopic signals<sup>15,21,24</sup> and isotope fractionation during decomposition and mineralization.<sup>25–28</sup> Nevertheless, this technique has been successfully used to document vegetation changes in a variety of locations worldwide.<sup>13,14,21,26,29–34</sup>

## Study sites and sampling strategy

The sites sampled in this study are located in the forests of Hluhluwe-Umfolozi Game Reserve, KwaZulu-Natal (28°00'S; 31°43'E). Soil samples were taken from three flat and three steeply sloping forested areas. All sites were covered by a continuous, tall forest canopy and were located away from the forest margins.

Soil was collected down a smooth profile of a soil pit. Soil pits were used to minimize the chance of sampling areas that had undergone recent bioturbation. Leaf litter was collected from directly above the soil pit before digging. Roughly 800 g of soil was collected at each depth. Pits were dug to a depth of 55 cm. Soil was sampled from depths of 0–10 cm, 15–25 cm, 30–40 cm and 45–55 cm.

## Analytical procedures

Sample preparation followed procedures set out in previous studies of soil organic matter.<sup>19,30,31</sup> Surface litter samples were oven-dried at 80°C and then ground in an electric mill to a fine powder. Soils were air-dried and passed through a 2-mm sieve to remove stones and loose organic matter. From every sample, a 200 g subsample was ground in a mortar and pestle. A further 10 g subsample was then treated with 10% HCl (10 M) overnight to dissolve away any carbonates. During this procedure, loose organic matter was also floated off. The samples were then rinsed and dried at 105°C overnight. Loose aggregates formed in the drying process were broken up. Depending on organic content, between 0.04 and 0.10 mg of sample was loaded into a mass spectrometer and combusted online. Isotopic ratios are expressed relative to the Pee Dee Belemnite Standard [ $\delta^{13}\text{C} = ((R_{\text{sample}} / R_{\text{PDB}}) - 1) \times 1000$ ], where R is the <sup>13</sup>C/<sup>12</sup>C ratio].

## Results

Samples from both flat and sloping locations indicated a trend from C<sub>3</sub> vegetation to C<sub>4</sub> vegetation with increase in depth (Fig. 1). The surface litter for all profiles was indicative of C<sub>3</sub> vegetation. The δ<sup>13</sup>C of the top 22.5 cm of SOM ranged between 24.1–22.7‰. The δ<sup>13</sup>C of SOM below that ranged from 19.1–14.8‰. Differences between the top to the bottom of the soil profile were 7.2‰ (slopes) and 9.3‰ (level ground) (Table 1).

## Discussion

There is evidence of a general trend from C<sub>4</sub>-dominated vegetation at some stage in the past to C<sub>3</sub>-dominated vegetation at present in the Hluhluwe Forest and environs. The profiles show a strong trend from C<sub>3</sub> covering vegetation and C<sub>3</sub>-derived SOM in the surface layers (0–25 cm) to organic material with a large C<sub>4</sub> component in deeper soil (30–55 cm) (Fig. 1). The <sup>13</sup>C-enriched soil levels at depth were seen in all soil profiles sampled in the Hluhluwe region. The deepest level sampled had δ<sup>13</sup>C values ranging from –19.1‰ to –14.8‰, which are typical of arboreal savanna<sup>21</sup> and tussock grasslands.<sup>19</sup> Thus it seems that the area now covered by forest was previously a grassland or grass-dominated savanna.

This raises the question of how old these forests are. The generally accepted view has been that of Acocks,<sup>1</sup> who proposed that forest was the climax vegetation in the KwaZulu-Natal

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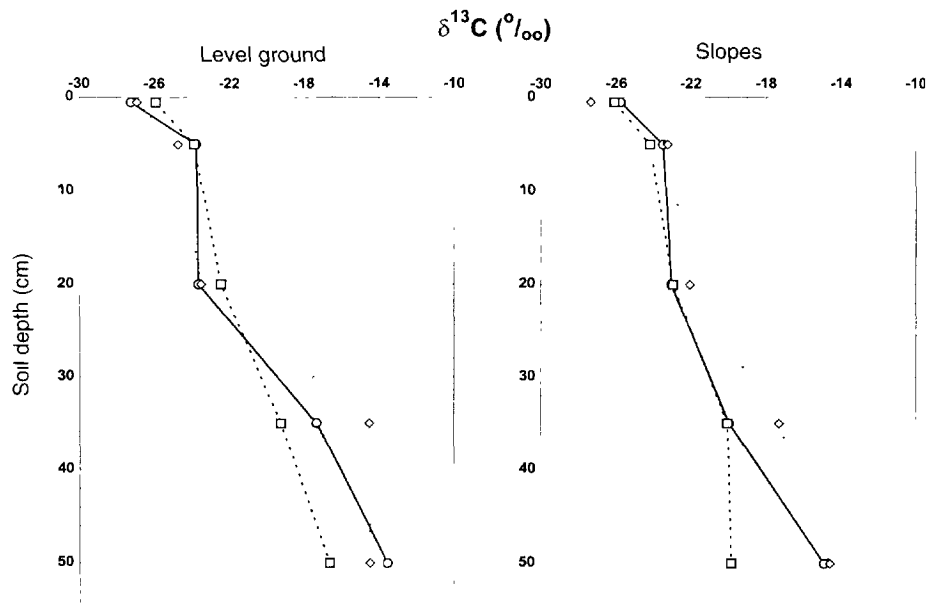
region. Acocks<sup>1</sup> postulated that grasslands in this region were 'false' grasslands, created by the burning and clearing of forests by Iron Age farmers. This hypothesis has been challenged before.<sup>6,7,35,36</sup> The trends from the Hluhluwe soil profiles support the hypothesis of Ellery and Mentis,<sup>9</sup> that the grasslands are in fact much older, and the forests consequently younger, than initially thought by Acocks.<sup>1</sup>

We do not at present have a date for this shift, as dating SOM is fraught with problems. This is mainly due to the methodological difficulty associated with <sup>14</sup>C dating of SOM, the differential rates of decomposition<sup>37</sup> and vertical migration of the various size fractions of organic material.<sup>38</sup> Using charcoal found at various depths in the profile to date SOM<sup>21</sup> also presents problems as bioturbation may result in younger fragments being placed deeper than older material. Desjardins *et al.*<sup>21</sup> found no correlation between depth and charcoal <sup>14</sup>C-age. Thus it is difficult to obtain an accurate age for SOM. Nevertheless, rough estimates can be obtained using the techniques mentioned above. This is an avenue to be pursued in future research.

**Causes of recent C<sub>4</sub> to C<sub>3</sub> vegetation shifts**

The changes in vegetation at Hluhluwe are not unique; there have been several reports of such a shift worldwide.<sup>21,29,30,34,39</sup> It seems that after the global expansion of C<sub>4</sub> ecosystems at the end of the Miocene,<sup>3-5</sup> a similarly widespread transformation from C<sub>4</sub>- to C<sub>3</sub>-dominated systems occurred in the Holocene.<sup>9,21,29,30,34,39</sup> The recent expansion of C<sub>3</sub> vegetation continues in the present and local climate change and land-use patterns, involving fire regimes and grazing practices, have been invoked to explain these trends.<sup>40</sup> However, the widespread nature of the Miocene and Holocene shifts suggests that a global cause, possibly the increase in global CO<sub>2</sub> levels, is responsible for these vegetation changes.

Several studies have indicated that CO<sub>2</sub> levels are responsible for transformations between C<sub>4</sub>- and C<sub>3</sub>-dominated ecosystems.<sup>39,41,42</sup> Low CO<sub>2</sub> concentrations are thought to lead to C<sub>4</sub>-dominated ecosystems.<sup>3</sup> An increase in atmospheric CO<sub>2</sub> should thus favour the establishment of woody (C<sub>3</sub>) plants. The increase in global CO<sub>2</sub> concentrations from ~180 ppm (18 000



**Fig. 1.** Profiles of  $\delta^{13}\text{C}$  of soil organic matter from level and steeply sloping ground in the forests of the Hluhluwe-Umfolozi Game Reserve. Individual curves represent replicate results of separate cores.

years ago) to ~275 ppm (10 000 years ago) corresponds well with the decrease in  $\delta^{13}\text{C}$  signals<sup>39</sup> and this may in part be due to anthropogenic fires.<sup>9</sup> The most recent increase in post-industrial atmospheric CO<sub>2</sub> to 360 ppm<sup>43</sup> may account for the current woodland expansion witnessed globally.

Whatever the cause, this study indicates that grasslands were the precursor to forest vegetation in the Hluhluwe region and that forests are the more recent vegetation cover. It will be interesting to see if further studies support this hypothesis.

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**Table 1** Mean ( $\pm$ s.d.,  $\delta^{13}\text{C}$  values (‰) of organic matter at each depth in the soil profiles from Hilltop Forest, Hluhluwe Nature Reserve. 'Level' represent areas of level ground within the forest. 'Slopes' represent steeply sloping forest areas. Overall shift in  $\delta^{13}\text{C}$  from shallowest to deepest soil level is shown.

Depth (cm)	Level (n = 3)	Slopes (n = 3)
surface litter	-26.7 $\pm$ 0.7	-26.4 $\pm$ 0.8
0-10	-24.1 $\pm$ 0.5	-23.6 $\pm$ 0.5
15-25	-23.2 $\pm$ 0.7	-22.7 $\pm$ 0.5
30-40	-17.0 $\pm$ 2.4	-19.1 $\pm$ 1.6
45-55	-14.8 $\pm$ 1.6	-16.4 $\pm$ 2.9
$\delta^{13}\text{C}$ shift down the soil profile	9.3	7.2

- 1 Acocks J PH (1953) Veld types of South Africa. *Mem. Bot. Surv. S. Afr.* 28, 1-128
- 2 Feely J M (1980) Did Iron Age man have a role in the history of Zululand's wilderness landscapes? *S. Afr. J. Sci.* 76, 150-151
- 3 Cerling TE, Wang Y and Quade J (1993) Expansion of C<sub>4</sub> ecosystems as an indicator of global ecological change in the late Miocene. *Nature* 361, 344-345
- 4 Cerling TE, Harris J M, MacFadden B J, Leakey M G, Quade J, Eisenmann V and Ehleringer J R (1997) Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389, 153-158
- 5 Quade J and Cerling TE (1995) Expansion of C<sub>4</sub> grasses in the Late Miocene of northern Pakistan: evidence from stable isotopes in palaeosols. *Palaeogeography, Palaeoclimatology, Palaeoecology* 115, 91-116
- 6 Ellery W.N. and Mentis M.T (1992) How old are South Africa's grasslands? In *Nature and Dynamics of Forest-Savanna Boundaries*, eds FA Furlley, J Proctor and J A Ratter, chap. 14, pp. 283-292. Chapman and Hall, London
- 7 Meadows M E and Linder H P (1993) A paleoecological perspective on the origin of afro-montane grasslands. *J. Biogeogr.* 20, 345-355
- 8 Ehleringer J R, Cerling TE and Helliker B R (1998) C<sub>4</sub> photosynthesis, atmospheric CO<sub>2</sub> and climate. *Oecologia* 112, 285-299
- 9 Bird M I and Cali J A (1998) A million year record of fire in Sub-Saharan Africa. *Nature* 394, 767-769
- 10 O'Leary M H (1993) Biochemical basis of carbon isotope fractionation. In *Stable Isotopes and Plant Carbon-Water Relations*, eds J R Ehleringer, A E Hall and G D Farquhar, chap. 3, pp. 19-28. Academic Press, San Diego
- 11 Boutton TW (1991) Stable carbon isotope ratios of natural materials. II. Atmospheric, terrestrial, marine and freshwater environments. In *Carbon Isotope Techniques*, eds D C Coleman and B Fry, pp. 173-185. Academic Press, San Diego
- 12 O'Leary M H (1988) Carbon isotopes in photosynthesis. *Bioscience* 38, 325-336
- 13 Guillet B, Favre P, Mariotti A. and Khobzi J (1988) The <sup>14</sup>C dates and <sup>13</sup>C/<sup>12</sup>C ratios of soil organic matter as a means of studying the past vegetation in intertropical regions: examples from Colombia (South America). *Palaeogeography, Palaeoclimatology, Palaeoecology* 65, 51-58

- 14 Stock WD, Bond WJ, and Le Roux D (1993) Isotope evidence from soil carbon to reconstruct vegetation history in the south western Cape Province *S Afr J Sci* **89**, 153–154
- 15 Boutton TW (1996) Stable carbon isotope ratios of soil organic matter and their use as indicators of vegetation and climate change. In *Mass Spectrometry of Soils*, eds T.W. Boutton and S. Yamasaki, pp 47–82. Marcel Dekker, New York
- 16 Wedin DA, Tieszen LL, Dewey B and Pastor J. (1995). Carbon isotope dynamics during grass decomposition and soil organic matter formation *Ecology* **76**, 1383–1392
- 17 Balesdent J, Wagner G.H. and Mariotti A (1988) Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance *Soil Sci Soc Am J* **52**, 118–124.
- 18 Bird MI and Pousa P (1997) Variations of  $\delta^{13}\text{C}$  in the surface soil organic carbon pool *Global Biogeochemical Cycles* **11**, 313–322.
- 19 Witt G B (1997) How the West was once reconstructing historical vegetation change and monitoring the present using carbon isotope techniques University of Queensland, Gatton.
- 20 Mariotti A and Balesdent J. (1990)  $^{13}\text{C}$  natural abundance as a tracer of soil organic matter turnover and paleoenvironment dynamics *Chem Geol* **84**, 217–219
- 21 Desjardins T, Carneiro A., Mariotti A, Chauvel A. and Girardin C (1996) Changes of the forest savanna boundary in Brazilian Amazonia during the Holocene revealed by stable isotope ratios of soil organic carbon *Oecologia* **108**, 749–756
- 22 Bird MI, Chrvas A.R. and Head J. (1996). A latitudinal gradient in carbon turnover times in forest soils *Nature* **381**, 143–146
- 23 February E and Van der Merwe NJ (1992). Stable carbon isotope ratios of wood charcoal during the past 4000 years: anthropogenic and climatic influences *S Afr J Sci* **88**, 291–292
- 24 Le Roux D, Stock WD., Bond WJ and Maphanga D. (1996) Dry mass allocation, water use efficiency and  $\delta^{13}\text{C}$  in clones of *Eucalyptus grandis*, *E. grandis* × *camaldulensis* and *E. grandis* × *nitens* grown under two irrigation regimes *Tree Physiol* **16**, 497–502
- 25 Balesdent J, Mariotti A and Guillet B. (1987) Natural  $^{13}\text{C}$  abundance as a tracer for soil organic matter dynamics studies *Soil Biol Biochem* **19**, 25–30
- 26 Mariotti A and Peterschmitt E. (1994) Forest savanna ecotone dynamics in India as revealed by carbon isotope ratios of soil organic matter *Oecologia* **97**, 475–480
- 27 Becker-Heidmann P and Scharpenseel H W (1992). The use of the natural  $^{14}\text{C}$  and  $^{13}\text{C}$  in soils for studies on global climate change. *Radiocarbon* **34**, 535–540
- 28 Demes P (1980) The isotopic composition of reduced carbon. In *Handbook of Environmental Isotope Geochemistry Vol 1 The Terrestrial Environment*, eds P Fritz and J Ch Fontes, pp 329–406. Elsevier, New York
- 29 Ambrose S H and Sikes N E (1991). Soil carbon isotope evidence for Holocene habitat change in the Kenya Rift Valley *Science* **253**, 1402–1405
- 30 Pessenda L C R, Aravena R, Melfi A.J., Telles E.C.C., Boulet R., Valencia E.P.E. and Tomazello M. (1996). The use of carbon isotopes ( $^{13}\text{C}$ ,  $^{14}\text{C}$ ) in soil to evaluate vegetation changes during the Holocene in central Brazil. *Radiocarbon* **38**, 191–201
- 31 Bond WJ, Stock WD and Hoffman M.T. (1994). Has the Karoo spread? A test for desertification using carbon isotopes from soils *S Afr J Sci* **90**, 391–397
- 32 McPherson G R, Boutton TW and Midwood A J. (1993). Stable carbon isotope analysis of soil organic matter illustrates vegetation change at the grassland/woodland boundary in southeastern Arizona, USA. *Oecologia* **93**, 95–101
- 33 DeLaune R D. (1986) The use of  $\delta^{13}\text{C}$  signature of C-3 and C-4 plants in determining past depositional environments in rapidly accreting marshes of the Mississippi River deltaic plain, Louisiana, U.S.A *Chemical Geology (Isotope Science Section)* **59**, 315–320
- 34 Connin S.L., Virginia R A and Chamberlain C P (1997) Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion. *Oecologia* **110**, 374–386
- 35 Feely J M (1985) Smelting in the Iron-Age of Transkei *S Afr J Sci* **81**, 10–11
- 36 Feely J M (1987) *The Early Farmers of Transkei, Southern Africa before AD 1870* British Archaeological Reports, Oxford.
- 37 Vitousek P.M. and Sanford R.L. (1986) Nutrient cycling in moist tropical forest. *Ann Rev Ecol Syst* **17**, 137–167
- 38 Hoffman M T, Bond WJ and Stock WD (1995) Desertification of the eastern Karoo, South Africa: conflicting palaeoecological, historical and soil isotope evidence. *Environmental Monitoring and Assessment* **37**, 159–177
- 39 Cole D R and Monger H.C (1994). Influence of atmospheric  $\text{CO}_2$  on the decline of  $\text{C}_4$  plants during the last deglaciation *Nature* **368**, 533–536
- 40 Archer S, Schimel D.S. and Holland E.A (1995) Mechanisms of shrubland expansion land use, climate or  $\text{CO}_2$  *Climatic Change* **29**, 91–99
- 41 Ehleringer J.R., Sage R F, Flanagan L B and Pearcy R W (1991) Climate change and the evolution of  $\text{C}_4$  photosynthesis. *Trend Ecol Evolut.* **6**, 95–99
- 42 Street-Perrott FA, Huang Y and Perrott R.A. (1997). Impact of lower atmospheric carbon dioxide on tropical mountain ecosystems *Science* **278**, 1422–1426
- 43 Farquhar G D (1997) Carbon dioxide and vegetation *Science* **278**, 1411

## Antimicrobial resistance of *Salmonella* isolates associated with retail chicken and a poultry abattoir

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**Animal feed is increasingly being supplemented with antibiotics to reduce the risk of epidemics in animal husbandry. This practice could lead to the selection of antibiotic-resistant microorganisms. The aim of this study was to determine the proportion of antibiotic-resistant *Salmonella* species present on retail and abattoir chicken in South Africa. *Salmonella* isolates were tested for resistance to amikacin, ceftazidime, chloramphenicol, gentamicin, streptomycin, tetracycline and trimethoprim-sulfamethoxazole. A large proportion of the bacterial strains displayed multiple antibiotic resistance (MAR). A larger degree of resistance to most antibiotics was displayed by the isolates from the abattoir samples. The incidence of MAR pathogenic bacteria was also higher in the abattoir isolates. These results imply that food-related infections due to *Salmonella* contamination will be relatively difficult to treat.**

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Global trends indicate progressive increases in the number of antibiotic-resistant *Salmonella* strains in humans and farm animals. The liberal administration of antibiotics in hospitals and the widespread abuse of antibiotic supplements in the meat industries has led to the emergence and persistence of resistant strains.<sup>1–3</sup>

Antibiotics have proved to be very effective in poultry husbandry practices. These practices include raising livestock in overcrowded batteries at optimal temperature and low light intensity to enhance growth rates and mass increases, and shortening the production cycles. Subtherapeutic doses of antimicrobials are administered routinely via feeds and water to raise the feeding efficiency and rate of weight gain in cattle, pigs and poultry.<sup>4–8</sup> The enhanced rate of weight gain has consequently led to a large increase in the use of antibiotics.<sup>2,8,9</sup> Animals are also fed carcass meal, edible plastic, sewage, petrochemical residues and excrement. These feed items are heavily contaminated with infectious bacteria so that additional antibiotics are widely used on poultry farms to suppress the outbreak of epidemics.<sup>6,10</sup> In the United States alone, seven million kilograms of antibiotics is administered to farm animals annually, of which approximately 40–50% is via livestock feed.<sup>3,7,9,11</sup>

Claims that the practice of dosing animals with subtherapeutic amounts of antibiotics promotes the development of antimicrobial resistance of pathogens have been increasing.<sup>7,11–13</sup> Moreover, the literature provides evidence for the emergence of pathogenic bacteria resistant to antibiotics, that could threaten human health.<sup>7,9,11,14</sup>

The genetic determinants of antibiotic resistance are encoded in the bacterial chromosome or in cytoplasmic plasmids.<sup>15</sup> Resistance (R) plasmids are autonomous and self-replicating fragments of DNA whose transcription and products confer resistance