nature portfolio

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# Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

| n/a | Confirmed |
|---|---|
| ☐ | ☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☒ | ☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☒ | ☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

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| Data collection | Microsoft Excel version 16.99.2 |
| Data analysis | Data visualisation was performed in R (v4.2.1; R Core Team, 2022) using the ggplot2 package for plotting, with additional formatting provided by showtext for font rendering and theme_minimal() for consistent aesthetics. |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

## Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The supplementary materials for this study include growth ring width measurements from modern wood samples, ring widths modelled from charcoal using experimentally determined shrinkage factors, and detailed calculations of seasonal $\delta^{13}C$ amplitude ($\Delta\delta^{13}Cmeas$). These datasets are available via the figshare repository (see Data Availability statement).

Data Availability Statement
All data supporting the findings of this study are publicly available in the figshare repository: https://doi.org/10.6084/m9.figshare.29460170.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

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| Reporting on sex and gender | *Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.*<br>*Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.*<br>*Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.* |
| Reporting on race, ethnicity, or other socially relevant groupings | *Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).*<br>*Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)*<br>*Please provide details about how you controlled for confounding variables in your analyses.* |
| Population characteristics | *Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."* |
| Recruitment | *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.* |
| Ethics oversight | *Identify the organization(s) that approved the study protocol.* |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

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| Study description | This study employed a factorial design to test the effects of seasonal rainfall (winter, summer, year-round), taxonomic group (Protea (angiosperm) and Podocarpus (gymnosperm), and material type (modern wood and experimentally carbonised wood from the same increment core) on intra-ring carbon isotope variability. For each combination of rainfall zone and taxonomic group, modern wood samples were collected from individual trees from each major rainfall zone. One increment core was extracted from the Protea and Podocarpus trees chosen to reflect that zone. The increment core was bisected and charcoal was produced from one half in a laboratory muffle furnace under controlled conditions. Intra-ring $\delta^{13}C$ values were obtained by serial subsampling along growth rings. These data were then compared to carbon isotope variability modelled from seasonal rainfall data for each respective rainfall zone, allowing assessment of how carbonisation affects $\delta^{13}C$ seasonal signals and the applicability of modern analogues to archaeological specimens. |
| Research sample | Protea:<br>Summer: paired wood and charcoal samples from the same Protea roupelliae tree<br>Year-Round: paired wood and charcoal samples from the same Protea nitida tree<br>Winter: paired wood and charcoal from the same Protea nitida tree, plus only wood from another Protea nitida tree at a different site.<br><br>Podocarpus:<br>Summer: only charcoal from a Podocarpus latifolius tree<br>Year-Round: paired wood and charcoal samples from the same Podocarpus latifolius tree<br>Winter: only charcoal from a Podocarpus elongatus tree<br><br>We sampled Protea and Podocarpus from each major rainfall zone to capture how seasonal rainfall patterns are reflected in their carbon isotope signatures across different plant types, material types, and seasonal climates. |

Sampling strategy

Increment cores were collected using a 1.2 mm increment borer, larger than typically used, to ensure sufficient material for bisecting the core and producing charcoal from one half, as well as for high-resolution serial sampling. Only one core was taken per tree, which is uncommon in dendrochronology and dendroclimatology, where replication (multiple cores per tree, and multiple trees per site) is prioritised. However, this study served as a preliminary test of the model in Southern Africa and on charcoal. Replication was addressed at the level of rainfall zones, which served as the primary scale of analysis, rather than at the level of individual sites, with 2–3 wood or charcoal samples representing each zone.

Data collection

In the field, metadata for each extracted increment core were clearly written on the sample bag surface. Recorded information included date, time, tree coordinates, species, and site name. These details, along with additional notes, were also logged in a field notebook. The data were subsequently transferred to a Microsoft Excel spreadsheet for organisation.
In the laboratory, microscope images of each sample were captured, and measurements of mass, width, and length for both wood and charcoal samples were taken and recorded in a laboratory notebook. During high-resolution serial subsampling, the distance from the bark (in millimeters) was documented on a dedicated recording form.
Uncalibrated $\delta^{13}C$ values were initially compiled in an Excel spreadsheet, where calibration was also performed. Gemma D. Poretti was responsible for data recording throughout both fieldwork and laboratory procedures.

Timing and spatial scale

2021 Field trips:
9-13 August - Swartberg, Western Cape (year-round zone)
3 September - half-day trip to Camps Bay, Western Cape (winter zone)
This marked the very start of the project, where only the winter and year-round zones were analysed. The summer rainfall zone was included later in the analysis.

2022 Field trips:
7-9 September 2022 - Montagu Pass, Western Cape (year-round zone)
4-6 December 2022 - Theronsberg and Gifberg, Western Cape (winter zone)

2023 Field trips
13-15 January 2023 - Giant's Castle, KwaZulu-Natal (summer zone)

These sampling sites ensured that all major rainfall zones were represented in the analysis. The gaps between sampling dates reflect breaks in the University of Cape Town's teaching calendar, which allowed Vincent J. Hare to accompany Gemma D. Poretti in the field. Additionally, site selection was iterative, initially analysing what was available, then later selecting additional sites as the project progressed.

Data exclusions

One charcoal sample from the winter rainfall zone (Gifberg; GB1_ch) was processed and isotopically analysed but subsequently excluded from the final analysis. The exclusion criteria are explicitly detailed in the Methods section and further discussed, with reference to GB1_ch, in the Discussion. This sample exhibited very narrow growth rings and an absence of meaningful $\delta^{13}C$ variation, which prevented application of the model. We interpret these features as a result of the summer drought characteristic of the Mediterranean-type climate, coupled with baseline aridity, leading to irregular and truncated growth and resulting in gaps within the $\delta^{13}C$ record for this individual. Additionally, carbonisation-induced shrinkage further reduced the available material for subsampling, exacerbating the limitations brought on by the narrow rings.
The winter zone Protea nitida charcoal sample (TB2) was excluded due to instrumental error (SD > 0.25), and this sample was entirely excluded from the manuscript.

Reproducibility

Sampling was standardised using the same 1.2 mm increment borer, with precise tree coordinates recorded for each site. Early analyses (2021) of Camps Bay wood and charcoal and Swartberg charcoal preceded standardised lab protocols: embedding samples in plaster of Paris to stabilise them, and measuring distance from bark were later added to improve consistency. All laboratory work was performed by Gemma D. Poretti, and detailed protocols were documented throughout the project's development. Isotopic analyses were calibrated using consistent in-house standards, which are regularly calibrated against international IAEA standards. The winter zone Protea nitida charcoal sample (TB2) was excluded due to instrumental error (SD > 0.25). All other sampling and analyses were successfully repeated.

Randomization

Sampling was not random. Samples were grouped according to rainfall zone, taxon (angiosperm or gymnosperm) and, later, material type (wood or charcoal). To control for taxonomic variation, the same genus was sampled across all rainfall zones: Protea for angiosperms and Podocarpus for gymnosperms. In the field, we sampled only from protected areas (parks and reserves), avoided roads, waterbodies, and closed canopy areas, and preferentially collected from upslope areas, to reduce other possible effects on $\delta^{13}C$ variability. Juvenile trees were not sampled, though we note that the summer rainfall zone Podocarpus specimen appeared relatively old. Yet no trees were formally aged or dated so this remains uncertain. While we strongly endeavoured to control covariates, some limitations must be acknowledged. Firstly, sampling height about ground was not measured or formally recorded, though care was taken to avoid sampling from too close to the ground or too high on the bark. Secondly, the season of field sampling was not consistent due to logistical constraints.

Blinding

Blinding was not implemented. The rainfall zone, genus, and material type were known during the interpretation of the $\delta^{13}C$ data, including the identification of isotopic peaks, troughs, and amplitudes. While it would have been possible to blind the isotope patterns during amplitude calculations by analysing them without metadata and comparing results later, this was not done. Given the exploratory and iterative nature of the study and the small dataset, blinding was not considered necessary.

Did the study involve field work? ☒ Yes ☐ No

## Field work, collection and transport

Field conditions

Field sampling was conducted across South Africa's major rainfall zones, with each site visited in a different season. All sampling took

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| Field conditions | place on clear days with no rainfall. Samples were extracted using a 1.2mm increment borer, and one sample was taken per tree.<br>Site-specific details are as follows:<br>Camps Bay: 03/09/2021, Spring (end of wet season)<br>Theronsberg Pass: 05/12/2022, Summer (dry season)<br>Gifberg Pass: 06/05/2022, Summer (dry season)<br>Swartberg Pass: 10/08/2021, Winter<br>Montagu Pass: 09/09/2022, Spring (rainfall peak)<br>Giant's Castle: 15/01/2023, Summer (wet season) |
| Location | Winter Rainfall Zone, Western Cape Province:<br>Camps Bay (33°57'13'' S 18°22'45'' E)<br>Theronsberg Pass (33°17'7'' S 19°27'50'' E)<br>Gifberg Pass (31°44'56'' S 18°47'22'' E)<br><br>Year-Round Rainfall Zone, Western Cape Province:<br>Swartberg Pass (33°21'54'' S 22°5'57'' E)<br>Montagu Pass (33°54'23'' S 22°25'9''E)<br><br>Summer Rainfall Zone, KwaZulu-Natal Province:<br>Giant's Castle Game Reserve (29°16'25'' S 29°31'19'' E and 29°15'51'' S 29°31'15'' E) |
| Access & import/export | Sampling in the Western Cape was conducted under permit from CapeNature (issued 24 June 2021: CN35-28-17503; and 3 August 2023: CN35-28-25863). Sampling in KwaZulu-Natal was conducted under permit from Ezemvelo KZN Wildlife (issued 13 June 2023: OP 2099/2023).<br>Samples were hand-carried and stored in the Stable Light Isotope Laboratory, Department of Archaeology, University of Cape Town. |
| Disturbance | Minimal disturbance was caused during fieldwork. Only small, non-destructive samples (increment cores, leaves, and a few branches) were collected from individual trees, posing minimal threat to tree health. Sampling was limited to the minimum number of specimens required for analysis, and care was taken to avoid damaging surrounding vegetation. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ Antibodies |
| ☒ | ☐ Eukaryotic cell lines |
| ☐ | ☒ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

| n/a | Involved in the study |
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| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Palaeontology and Archaeology

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| Specimen provenance | The two archaeological Proteaceae charcoal specimens analysed in this study were excavated from the Last Glacial Maximum deposits of Boomplaas Cave, in the Cango Valley, Western Cape, South Africa. Access to these samples was granted under the permit held by Justin Pargeter who is conducting a re-excavation of Boomplaas Cave as part of the Cango Valley Archaeology and Paleoscape Project. This study was carried out with his permission and in accordance with all relevant regulatory requirements. |
| Specimen deposition | The archaeological charcoal specimens analysed in this study were completely consumed during the destructive sampling process. |
| Dating methods | The charcoal specimens analysed in this study were previously radiocarbon dated (Pargeter et al. 2018). However, no new dating was performed as part of this research. |

☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

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| Ethics oversight | Ethical approval was not required for this study as it involved only archaeological charcoal samples and did not include human participants or animal subjects. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Plants

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| Seed stocks | *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.* |
| Novel plant genotypes | *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.* |
| Authentication | *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.* |