

Stable carbon isotope analysis on fossil *Cedrus* pollen shows summer aridification in Morocco during the last 5000 years

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ABSTRACT: Quantitative climate reconstructions from pollen typically rely on empirical relationships between pollen abundances or assemblages and climate, such as the modern analogue technique. However, these techniques may be problematic when applied to fossil sequences, as they cannot separate anthropogenic from climatic influence on pollen assemblages. Here, we reconstruct Mid- to Late Holocene summer aridity in the Middle Atlas, Morocco, using stable carbon isotope analysis of isolated fossil *Cedrus* pollen. This approach is based on well-documented plant physiological responses to moisture stress and is therefore independent of vegetation composition. We find that there has been a general long-term trend of increasing summer aridity in the region during the last 5000 years to the present day. The gradual decline of *Cedrus atlantica* forest in the Late Holocene follows this aridity trend. Additionally, we show how isolating a specific pollen type for carbon isotope analysis yields a robust climate signal, versus using pollen concentrates or bulk sediment. Our findings indicate that climate has become drier in the region and confirms the Mid- to Late Holocene aridification trend observed more widely in the western Mediterranean, using a novel proxy for this region with good potential for wider application in other environments.

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Introduction

The Middle Atlas region of Morocco lies at the convergence of Saharan and Atlantic air masses, yielding a semi-arid to sub-humid climate (Born *et al.*, 2008). Fossil pollen assemblages from sediment cores taken across the Middle Atlas have provided insights into the region's past environment and climate (e.g. Lamb *et al.*, 1989; Cheddadi *et al.*, 1998, 2015, 2019; Rhoujjati *et al.*, 2010; Nour El Bait *et al.*, 2014; Tabel *et al.*, 2016; Campbell *et al.*, 2017). A common pattern in these records is the virtual absence of *Cedrus* pollen in the Early Holocene, with the species generally becoming more prevalent from the Mid-Holocene.

The Middle Atlas is the largest core area of the North African *Cedrus atlantica* range despite deforestation during the recent millennia (Benabid and Fennane, 1994) and more recent dieback (Rhanem, 2011). The Mid-Holocene colonization of the Middle Atlas has been interpreted primarily as a signal of increased moisture availability (e.g. Cheddadi *et al.*, 1998; Campbell *et al.*, 2017; Zielhofer *et al.*, 2017a, 2019). However, other factors such as winter temperature (Touchan *et al.*, 2017), or ecological and edaphic conditions could have also influenced *Cedrus* expansion. Indeed, *C. atlantica* is a drought-tolerant species (but is sensitive to extreme drought; Aussenac, 1984; Rhanem, 2011) and is found growing in semi-arid mountain areas across North Africa (Fig. 1A), some of which have relatively low annual rainfall. Therefore, open questions remain about the role of

climate in the establishment, persistence and decline of *C. atlantica* which may be difficult to answer based on pollen abundance data alone.

Quantitative climate reconstructions based on pollen assemblages typically rely on analogue matching (e.g. Modern Analogue Technique, MAT) or transfer functions to derive climatic inferences. However, these require a training set of modern assemblages and well-constrained pollen–climate relationships. Where the composition of either the fossil or the modern assemblages is strongly influenced by non-climatic factors including ecological succession, fire and anthropogenic activity (tree felling, farming, grazing) the approach can be problematic (Wahl, 2004; Ohlwein and Wahl, 2012). Accordingly, the increased impact of anthropogenic activity evident in Morocco during the Late Holocene (Zapata *et al.*, 2013; Cheddadi *et al.*, 2015; Campbell *et al.*, 2017) which had widespread influence on vegetation composition, could potentially make pollen-based climate reconstructions using MAT unreliable, and other complementary approaches should be considered.

Climate reconstructions based on *C. atlantica* tree-rings should be less susceptible to anthropogenic influence. These show a multi-centennial scale pattern for the last ~900 years with drier winters driven by positive winter NAO during the Medieval Climate Anomaly (MCA) (Trouet *et al.*, 2009), wetter conditions during the Little Ice Age (LIA), and very recent dry conditions, with considerable interannual variability (Esper *et al.*, 2007; Touchan *et al.*, 2011). However, *C. atlantica* tree-ring-based studies cannot match the long-term perspectives offered by pollen studies, which could extend to at least the last 30 000 years in the Middle Atlas (e.g. Rhoujjati *et al.*, 2010).

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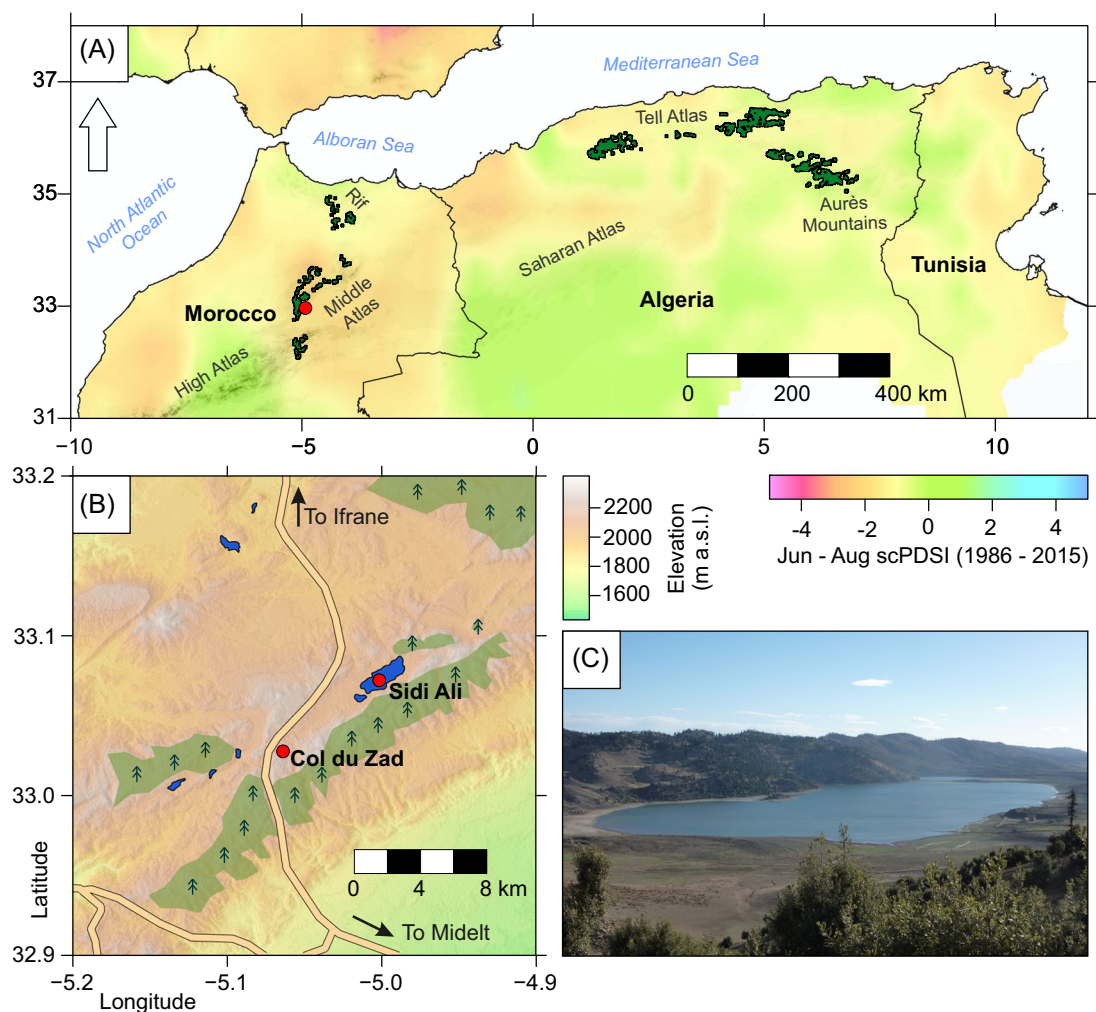


Figure 1. Study location showing (A) distribution of *Cedrus atlantica* trees in North Africa based on data from Farjon (1990), Sbabdji *et al.* (2009), Farjon and Filer (2013), Jihad (2017), and GLCC satellite imagery (U.S. Geological Survey, 2017). The map shows interpolated mean summer (June, July, August) aridity (scPDSI) for the period 1986–2015 based on CRU v3.24 datasets (Osborn *et al.*, 2017). (B) Location of the main study area showing the two core sites. (C) Photo of Lake Sidi Ali core site taken from the north-east looking south-west. [Color figure can be viewed at wileyonlinelibrary.com].

Here, we present the first stable carbon isotope analysis of isolated fossil *Cedrus* pollen from two sites in the Middle Atlas spanning the Mid- to Late Holocene. We demonstrate a methodology for the concentration and isolation of fossil pollen without the use of carbon-bearing chemicals. We also analyse pollen concentrates (organic residues rich in pollen) and bulk sediment samples from the same sample depths to evaluate the need for pollen isolation. *C. atlantica* is a climatically sensitive montane conifer endemic to North Africa of great biogeographical interest. *Cedrus* pollen are large ($59.1 \pm 4.0 \mu\text{m}$) and easily identifiable, facilitating their study for isotope analysis (Bell *et al.*, 2018). Furthermore, as an autumn pollinating species, pollen develops over the summer, making it possible to infer a summer climate signal (Bell *et al.*, 2017).

There are well-established relationships between the stable carbon isotope composition of plant material and plant physiology (Farquhar *et al.*, 1989). Photosynthesis allows plants to fix carbon (C); both ^{12}C and ^{13}C from CO_2 in the atmosphere through leaf stomata. Plants also regulate the rate of transpiration by changing the size of the stomata aperture to reduce water loss, allowing plants to adjust to different environmental conditions (Farquhar and Sharkey, 1982). In wetter conditions where stomata are fully open, $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ move freely between the atmosphere and leaf intercellular spaces. C fixation favours the lighter isotope (^{12}C) and intercellular $^{12}\text{CO}_2$ is readily replenished as it is fixed; hence discrimination against the heavier isotope is greater. In drier conditions, stomata close to reduce transpiration, restricting the

free movement of CO_2 . Consequently, C fixation is limited to CO_2 remaining in the leaf intercellular spaces, thereby increasing fixation of (or reducing discrimination against) the heavier isotope (^{13}C). Therefore, the ratio of ^{12}C to ^{13}C ($\delta^{13}\text{C}$) in plant tissues is dependent on environmental moisture availability at the time of C fixation. It is this relationship that allows a moisture availability signal to be reconstructed from $\delta^{13}\text{C}$ of plant material (Farquhar *et al.*, 1989; Dawson *et al.*, 2002; Diefendorf *et al.*, 2010).

As pollen $\delta^{13}\text{C}$ is highly correlated with parent leaf material $\delta^{13}\text{C}$ (Jahren, 2004), it can also be linked to environmental moisture availability. Early studies established that pollen $\delta^{13}\text{C}$ reflects C3/C4 photosynthetic pathways (Amundson *et al.*, 1997; Nelson *et al.*, 2006; Descolas-Gros and Schölzel, 2007). Studies have also demonstrated a moisture signal in pollen $\delta^{13}\text{C}$ of C3 plants (e.g. Loader and Hemming, 2004; Nelson, 2012; Griener *et al.*, 2013). For *C. atlantica*, we previously demonstrated a strong moisture availability signal in modern pollen, and a strong relationship between pollen and parent leaf material $\delta^{13}\text{C}$ (Bell *et al.*, 2017). While most studies have focused on modern pollen, Griener *et al.* (2013) also studied fossil pollen to reconstruct moisture availability. However, applications of stable carbon isotope analysis on fossil pollen remain rare.

Several studies have detected a Late Holocene aridification trend from ecological or hydrological records in the western Mediterranean (Jalut *et al.*, 2009; Carrión *et al.*, 2010; Jiménez-Moreno *et al.*, 2015). The parallel increase in human impact leaves open questions about the main drivers of vegetation

changes and stimulates the debate regarding the interaction of climatic and human impacts. Our summer aridity reconstruction allows us to evaluate the long-term aridity trends in the Middle Atlas from the Mid-Holocene to the present day, offering new climate insights based on pollen geochemistry.

Methods

Sample collection and preparation

Samples were obtained from sediment cores from two sites in the Middle Atlas, Morocco (Fig. 1B), including a deep perennial lake, Lake Sidi Ali (33.07 °N, 5.00 °E, 2080 m a.s.l.) (Campbell *et al.*, 2017; Zielhofer *et al.*, 2017a), and a shallow spring-fed wetland at Col du Zad (33.03 °N, 5.07 °E, 2148 m a.s.l.) (Campbell, 2017). The two sites are located within 5 km of each other and experience the same climate. The main wooded areas (including *Cedrus atlantica*) are located on the margins of Lake Sidi Ali (Fig. 1C), and a few kilometres from the Col du Zad core site. We hypothesize that while pollen assemblages can be influenced by local variability in the environmental setting, the isotope composition should be unaffected. The use of two sites allows us to test this and evaluate whether pollen isotope results from different sites can be integrated.

Sample depths were chosen to cover the Mid- to Late Holocene period when *Cedrus* forests were well established, with one sample from the Early Holocene where a first interval of *Cedrus* expansion occurs. To obtain sufficient pollen for analysis, each sample integrated up to 4 cm of material for Lake Sidi Ali and up to 2 cm of material for Col du Zad, representing ~20 and ~30 years, respectively (Campbell, 2017; Fletcher *et al.*, 2017).

Fossil pollen isolation

As traditional pollen preparation techniques (notably acetolysis) can alter the geochemical signal due to carbon-bearing chemicals (Loader and Hemming, 2004; Descolas-Gros and

Schölzel, 2007; Nelson, 2012), a new technique based on micro-sieving (Heusser and Stock, 1984; Brown *et al.*, 1989), sodium pyrophosphate washes (Bates *et al.*, 1978) and dense media separation (Nakagawa *et al.*, 1998; Campbell *et al.*, 2016; Fletcher *et al.*, 2017) was used to isolate pollen. The first stage involves concentration of the target pollen grains (summarized in Fig. 2 and described below), followed by the isolation (pollen picking) stage.

Up to 10 g of sediment was placed in a 50-mL centrifuge tube and disaggregated in 30 mL of 0.01 M sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_4$). Sediment was then sieved at 90 μm to remove coarse particles. The sub 90 μm material was washed by mixing with $\text{Na}_4\text{P}_2\text{O}_4$ and centrifuging for 3 min at 2200 r.p.m. and discarding the supernatant containing fine clays. This was repeated until the supernatant was clear, typically eight or more times. Samples were washed twice with deionized water (dH_2O) to remove the $\text{Na}_4\text{P}_2\text{O}_4$.

Samples were subsequently treated with 20 mL 10% HCl in a hot water bath for 15 min, or until effervescence stopped, to remove carbonates. Two density separations followed, the first using a sodium polytungstate (SPT) solution at 1.9 g cm^{-3} and a second separation using an SPT solution at 1.6 g cm^{-3} . The first separation aims to separate organic (<1.9 g cm^{-3}) and mineral matter, while the second aims to concentrate pollen material in the lighter fraction (1.6 g cm^{-3}). Each treatment involved centrifuging for 20 min at 1800 r.p.m. After the second density separation, the supernatant containing the light material was split into two clean 50-mL centrifuge tubes and topped with dH_2O . This was centrifuged for 3 min at 4500 r.p.m., the two pellets were recovered and recombined into a single tube. Splitting into two tubes ensures that the density of the solution is sufficiently low as to retain all pollen in the pellets. This was washed thoroughly with dH_2O to remove any remaining traces of SPT. The remaining sample was sieved at 32 μm to remove small pollen and other particles to concentrate the larger *Cedrus* pollen grains.

Pollen isolation was carried out on the concentrated pollen samples under a light microscope at 100 \times optical zoom using a mouth pipette as described by Mensing and Southon (1999). A small solution of the concentrated sample was diluted in

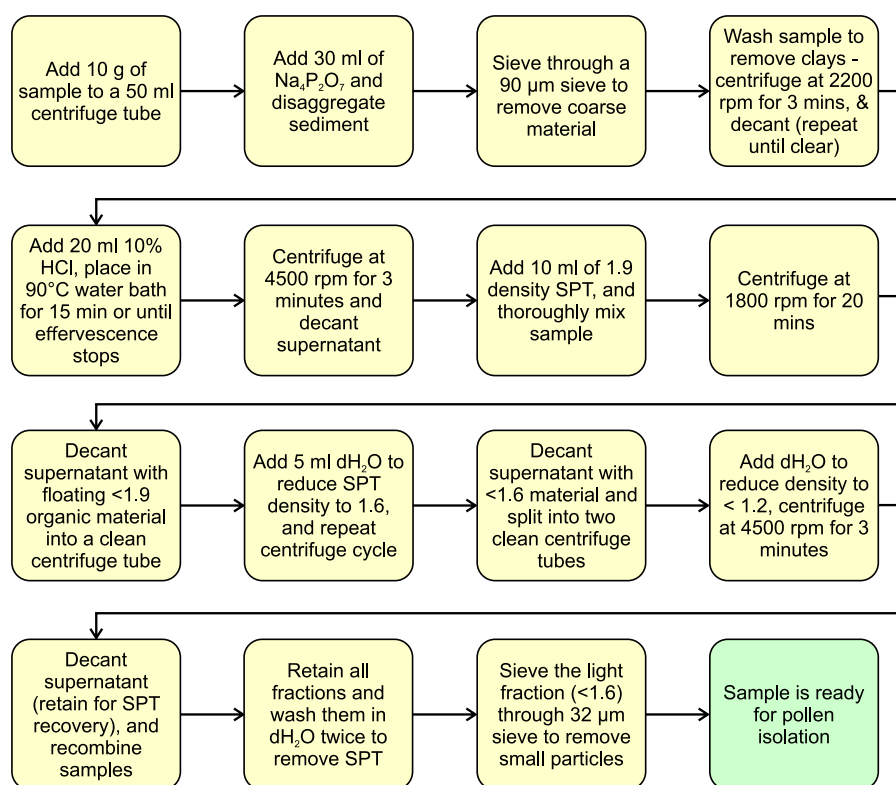


Figure 2. Simplified flow chart for the pollen concentration stage, for preparation of fossil *Cedrus* pollen samples for pollen isolation using micro-sieving and dense-media separation. [Color figure can be viewed at wileyonlinelibrary.com].

dH₂O and then transferred to a well slide using a micropipette (Fig. 3). *Cedrus* pollen grains were picked using the mouth pipette and transferred to a second well to isolate the grains from non-pollen material. Sometimes, other non-pollen material was transferred with the isolated grains into the second well. A final pick from the second well to be transferred into a small glass vial was used to remove this non-pollen material. Additional dilution with dH₂O was applied as necessary for the final pick.

To ensure the pollen samples contained enough carbon for accredited $\delta^{13}\text{C}$ results, a target number of 4000 grains was picked for each sample. Pollen picking time for each sample averaged 16 h, ranging from 12 to 21 h, with time taken largely dependent on the quantity of *Cedrus* pollen grains in the sample.

Pollen concentrates and bulk sediment

Preparation of pollen concentrates followed the method described above, except *Cedrus* pollen grains were not isolated from these samples. Bulk sediment was prepared by leaving in 30 mL 10% HCl for 24 h to remove carbonates, then washed twice with dH₂O.

Stable isotope analysis

Stable carbon isotope analysis was carried out by the NERC Life Sciences Mass Spectrometry Facility at CEH Lancaster. Samples were dried at 105 °C for 1 h and cooled in a desiccator, then weighed into small tin capsules for measurement. The isolated *Cedrus* pollen samples weighed between 0.040 and 0.075 mg. Capsules were combusted using an automated Eurovector elemental analyser coupled to an Isoprime Isotope Ratio Mass-Spectrometer (Elementar UK Ltd). An in-house working standard RefFLO (plain flour) was also analysed, resulting in a maximum analytical precision of 0.13‰ for isolated pollen, and 0.14‰ for pollen concentrates and bulk sediment. Data were normalized using in-house standards RefICE (cane sucrose) and RefGTAM (glutamic acid), calibrated annually against international standards (Sucrose-ANU – NIST 8542, and Glutamic acid – NIST 8573) and checked quarterly alongside the Wageningen proficiency testing samples within the International Plant Exchange (IPE) and International Soil Exchange (ISE) schemes. Three samples of each in-house standard are measured at the start and end of each sample run, used to construct a normalization calibration curve.

Sample size and preparation time

Theoretically, it is possible to analyse a sample containing a minimum of 5 μg carbon, although real world analysis often requires larger samples (Boutton, 1991). A series of tests using fresh *C. atlantica* pollen (~60% carbon) were run to determine

the minimum quantity required for analysis. A theoretical minimum for the elemental analysis isotope ratio mass spectrometry (EA-IRMS) setup at CEH is 15 μg carbon. However, it was found that this minimum resulted in shifts to the known $\delta^{13}\text{C}$ value of the reference material (sucrose) by 0.6‰, and the known $\delta^{13}\text{C}$ of the pollen sample by 0.2‰, while the in-house standard remained unchanged. At 20 μg carbon, results were more consistent with expected $\delta^{13}\text{C}$ values, but 30 μg carbon was found to be the 'optimal minimum'. As *C. atlantica* sporopollenin has a lower carbon content (~50% carbon) (Bell *et al.*, 2017), a target minimum weight of 60 μg of fossil pollen grains (or ~4000 grains) was required for analysis. The number of grains required for other pollen types and/or other laboratories is likely to vary.

This large number of pollen grains analysed has the benefit of providing a robust result that is representative of average environmental and climate conditions for the region. It may also be important to 'over-sample' due to potential losses of pollen grains between the preparation stages, e.g. transferring pollen grains from the glass vials to tin capsules for combustion inevitably results in small losses.

Data processing

All data and statistical analyses were carried out using R (R Core Team, 2016). For pure *Cedrus* pollen (modern and isolated fossil), $\delta^{13}\text{C}$ values were converted to discrimination ($\Delta^{13}\text{C}$) using the formula: $\Delta^{13}\text{C} (\text{‰}) = (\delta_A - \delta_B) / (1 + \delta_B \div 1000)$ where δ_A is the $\delta^{13}\text{C}$ of atmospheric CO₂, and δ_B is the $\delta^{13}\text{C}$ of the sample (Farquhar *et al.*, 1989); this conversion corrects for changes in the $\delta^{13}\text{C}$ of CO₂ over time (Schubert and Jahren, 2012). A second correction was made to the fossil pollen $\Delta^{13}\text{C}$ values to additionally account for the impact of changes in the concentration of atmospheric CO₂ (*p*CO₂ ppmv), as detailed in Schubert and Jahren (2015; 2018). Atmospheric CO₂ concentrations have varied from ~260 ppmv in the Mid-Holocene to the present day ~400 ppmv. These corrections are therefore important to allow robust comparison of fossil and modern isotopic values. Historical CO₂ $\delta^{13}\text{C}$ and *p*CO₂ ppmv values were obtained from ice core records (Francey *et al.*, 1999; Bauska *et al.*, 2015; Eggleston *et al.*, 2016). Full isotope data and corrections applied are available in the Supporting Information.

Aridity reconstruction

Aridity reconstructions were carried out using ordinary least squares regression, based on the relationship first established between the stable carbon isotope composition of modern *C. atlantica* sporopollenin and summer aridity in Bell *et al.* (2017). At the time of the original study, normalization of $\delta^{13}\text{C}$ results against the Wageningen proficiency testing schemes was not used by CEH. Therefore, to ensure compatibility between the modern and fossil isotope results, six modern

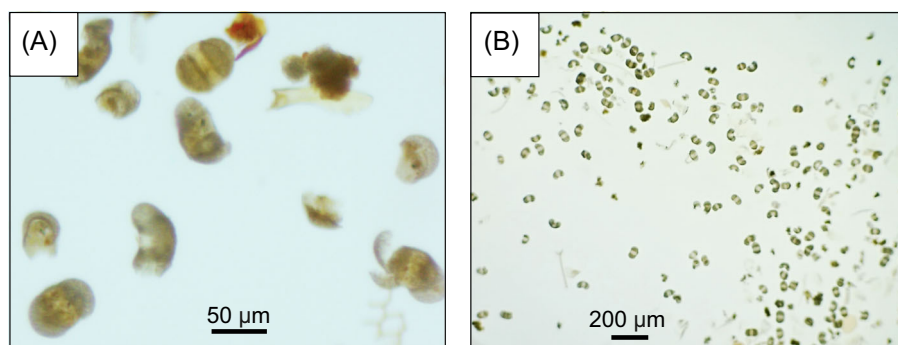


Figure 3. Photographs showing pollen concentrates under a Leica light microscope using the method described in this paper, before the pollen isolation stage, at: (A) 400 \times optical zoom, and (B) 100 \times optical zoom. *Cedrus* grains are easily identifiable and are ready to be isolated (picked) from these samples. [Color figure can be viewed at wileyonlinelibrary.com].

Table 1. Updated regression analysis showing the relationship between modern *Cedrus atlantica* sporopollenin (Bell *et al.*, 2017) and summer aridity (scPDSI), using normalized $\Delta^{13}\text{C}$ values based on the method described in this study.

Aridity dataset	R^2	p	RMSE	Bias	SE
CRU v3.24	0.72	0.0001	0.8224929	-0.000794732	0.11
Dai (2011)	0.74	0.0001	0.7904792	-0.01452961	0.14

C. atlantica pollen samples were re-analysed at CEH. We used the new $\delta^{13}\text{C}$ results (which differ by approximately -0.45‰) to apply a correction to the original data using the formula: $\delta^{13}\text{C}_{\text{corr}} = 1.0287 * \delta^{13}\text{C} + 0.2724$. We then re-ran the regression analysis to establish the relationship with aridity (Table 1).

Aridity data from the self-calibrating Palmer Drought Severity Index (scPDSI) were extracted from two gridded datasets: CRU v3.24 (Osborn *et al.*, 2017) and Dai (2011). Due to differences in the calculation of these datasets, the severity of reported aridity can vary (Van Der Schrier *et al.*, 2013). Although there is a stronger relationship with our isotope results and aridity data from Dai (2011), the CRU dataset is considered a more useful indicator of aridity in the region because it also accounts for snowmelt. Both sets of results are presented here for comparison with other published records. Due to the close proximity of the modern pollen sample sites (Bell *et al.*, 2017) in Morocco, and the low resolution of the gridded aridity data: 0.5×0.5 and $2.5 \times 2.5^\circ$ for CRU and Dai (2011), respectively, the scPDSI data for Morocco were interpolated using bilinear interpolation.

Results

Uncorrected $\delta^{13}\text{C}$ results

Stable carbon isotope analysis shows $\delta^{13}\text{C}$ has become less negative in isolated fossil *Cedrus* pollen during the last ~5000 years, from a peak of -24.5‰ at ~5100 cal a BP to -23.7‰ at 1150 cal a BP (Fig. 4A). At ~8200 cal a BP, $\delta^{13}\text{C}$ was -24.2‰ ; more negative than the most recent results but less negative than at 5100 cal a BP. The $\delta^{13}\text{C}$ values from the two different sites are closely aligned.

Pollen concentrates show site-specific differences between the $\delta^{13}\text{C}$ values, with Col du Zad having more negative $\delta^{13}\text{C}$ values compared to Lake Sidi Ali, with peak low values at -27.7‰ at ~5100 cal a BP (Fig. 4B). Lake Sidi Ali pollen concentrates yield similar $\delta^{13}\text{C}$ values to isolated fossil *Cedrus* pollen, while Col du Zad values differ by around -2.6‰ .

Bulk sediment also shows site-specific differences, with all Col du Zad $\delta^{13}\text{C}$ values around -28‰ , while Lake Sidi Ali $\delta^{13}\text{C}$ values are between -22‰ and -25‰ (Fig. 4C). The Lake Sidi Ali bulk sediment $\delta^{13}\text{C}$ values are also similar to isolated fossil *Cedrus* pollen. There is no overall trend in either the pollen concentrates or the bulk sediment results.

Cedrus pollen corrected $\Delta^{13}\text{C}$

In isolated fossil *Cedrus* pollen, discrimination has decreased during the last ~5000 years (Table 2; Fig. 5C). Discrimination peaked at $\Delta^{13}\text{C}$ 20.7‰ around 5100 cal a BP, which subsequently decreased by 1.1 to -19.6‰ around 1150 cal a BP, with a further 0.6‰ decrease during the last ~900 years to a present-day average of 19.0‰. At ~8200 cal a BP, $\Delta^{13}\text{C}$ was 20.5‰, which is higher than at the present day, but lower than the peak at ~5100 cal a BP. The $\Delta^{13}\text{C}$ values of isolated fossil *Cedrus* pollen samples from the two different sites are closely aligned (Fig. 5C).

Aridity reconstruction

Summer aridity reconstruction (Fig. 5D) shows there has been a long-term overall trend towards drier conditions in the Middle Atlas region during the last ~5000 years. The largest changes appear to occur around 5100 to ~4000 cal a BP, and again from ~1000 cal a BP to the present day. The severity of aridity appears higher when reconstructed using data from Dai (2011), although the pattern of aridity change is similar for both datasets. Present-day summer aridity is approximately 1 point lower (drier) on the scPDSI scale compared to conditions at ~5100 cal a BP. At ~8200 cal a BP, summer aridity is drier than at ~5100 cal a BP, but it is more humid than the Late Holocene period. The reconstruction shows that summers in the Middle Atlas have always been in a state of drought, although the severity of this drought has increased.

Discussion

Stable isotope composition of paired fossil material

The $\delta^{13}\text{C}$ values from pollen concentrates and bulk sediment are notably different between Col du Zad and Lake Sidi Ali, while isolated *Cedrus* pollen from the two sites are remarkably similar. Modern *Cedrus* pollen collected near Col du Zad and Lake Sidi Ali present statistically similar carbon isotopic ratios (Bell *et al.*, 2017). The closely aligned values in the fossil pollen data (Fig. 4A) are consistent with a common prevailing climate at these

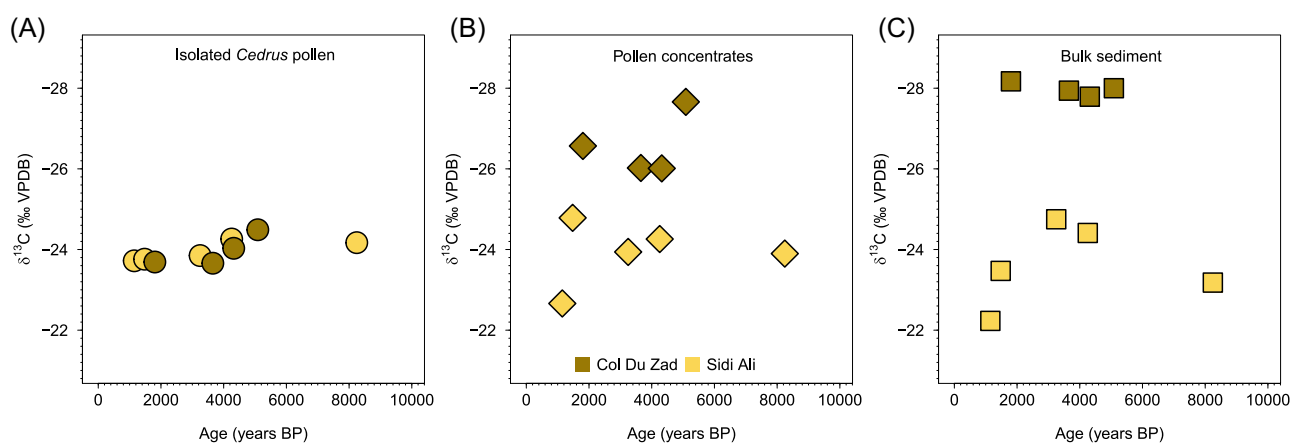


Figure 4. Uncorrected $\delta^{13}\text{C}$ results from: (A) isolated *Cedrus* pollen, (B) pollen concentrates and (C) bulk sediment, from Col du Zad (darker colour) and Lake Sidi Ali (lighter colour). More negative $\delta^{13}\text{C}$ values indicate wetter conditions. [Color figure can be viewed at wileyonlinelibrary.com].

Table 2. Results of stable carbon isotope analysis, including: updated modern sporopollenin results for Lake Sidi Ali and Col du Zad (Bell *et al.*, 2017), and fossil pollen (sporopollenin) results (this study). Corrected $\Delta^{13}\text{C}$ results are used for the aridity reconstruction.

Sample	Core depth (cm)	Median age (cal a BP)	Normalized $\delta^{13}\text{C}$ (‰)	Normalized $\Delta^{13}\text{C}$ (‰)	Corrected $\Delta^{13}\text{C}$ (‰)
SA-M	N/A	Present day	-26.10	18.23	19.72
CDZ-M	N/A	Present day	-27.51	19.72	18.23
SA-4	337–341	1148	-23.72	17.71	19.56
SA-6	462–466	1478	-23.76	17.76	19.64
CDZ-7	61–63	1805	-23.69	17.70	19.56
SA-9	822–828	3249	-23.85	17.94	19.84
CDZ-10	101–103	3654	-23.66	17.74	19.70
SA-11	976–978	4255	-24.26	18.37	20.36
CDZ-11	111–113	4320	-24.03	18.14	20.12
CDZ-12	121–123	5088	-24.49	18.62	20.68
SA-15	1418–1422	8244	-24.17	18.23	20.49

CDZ, Col du Zad; SA, Lake Sidi Ali.

closely located sites. In contrast, bulk sediment contains a mix of organic material of terrestrial and aquatic origin, including different pollen types, spores, algae, plant tissue, insect fragments and charcoal, which may respond differently to the prevailing climate. Similarly, pollen concentrates include multiple pollen types as well as aquatic microfossils, which are difficult to separate out fully (Fletcher *et al.*, 2017), thereby affecting the $\delta^{13}\text{C}$ values.

The greater inter- and intrasample variability in these sample types (Fig. 4B,C) probably results from the diversity of contributing species and mixing of terrestrial and aquatic carbon sources. We suggest that the strongly depleted isotopic values of pollen

concentrates and bulk samples from Col du Zad reflect a relatively high contribution of organic matter from abundant aquatic macrophytes (*Potamogeton*, *Ranunculus* spp.), and the spring-fed meadow of terrestrial hygrophytes (Cyperaceae, Poaceae spp.), consistent with $\delta^{13}\text{C}$ values for aquatic macrophytes without assimilation of bicarbonate (Keeley and Sandquist, 1992). In contrast, steep rocky slopes and lack of marginal plant communities (Zielhofer *et al.*, 2017a) may underline the similar isotopic values of the three sample types at Lake Sidi Ali.

Overall, we consider that the isotopic signal of pollen concentrates and bulk sediment will reflect the assemblage

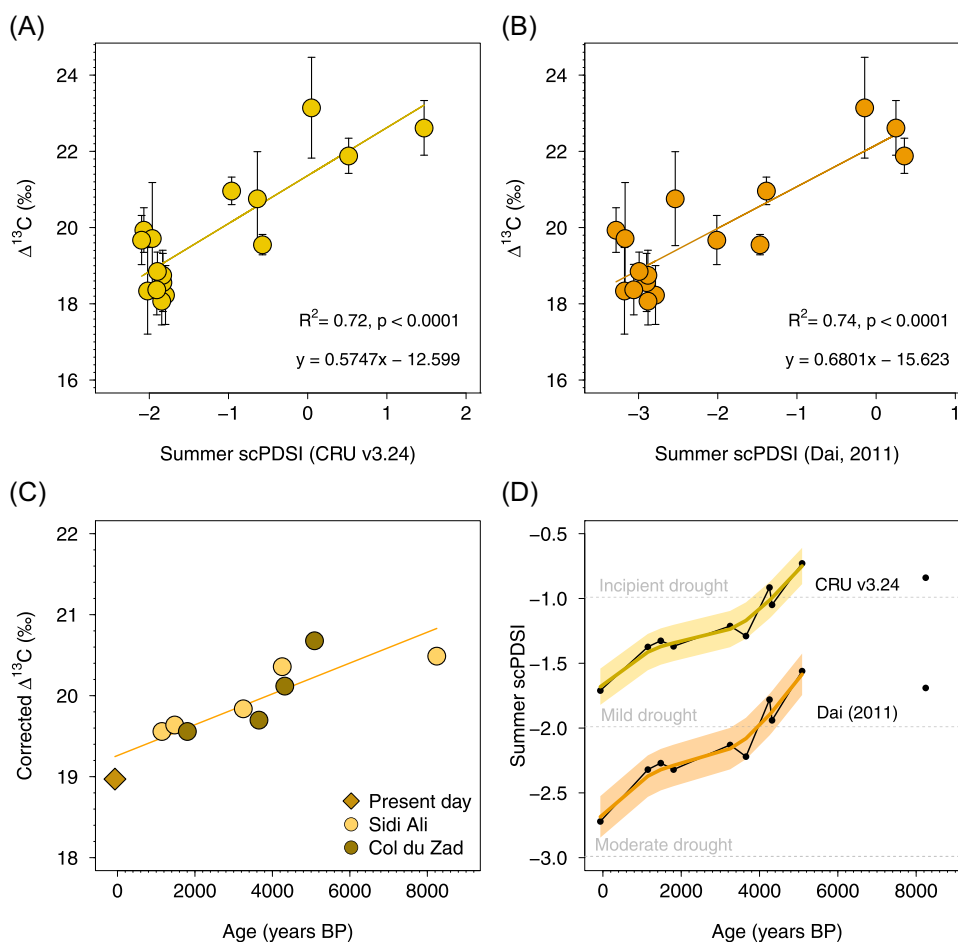


Figure 5. Relationships between modern *Cedrus atlantica* sporopollenin (Bell *et al.*, 2017) using normalized $\Delta^{13}\text{C}$ values (this study) and the self-calibrating Palmer drought severity index (scPDSI), based on: (A) CRU v3.24 scPDSI (Osborn *et al.*, 2017) and (B) Dai (2011) datasets. (C) Results of stable carbon isotope analysis on fossil *Cedrus* pollen corrected for changes in CO_2 $\delta^{13}\text{C}$ and CO_2 ppmv over time (Schubert and Jahren, 2012, 2015, 2018). (D) Reconstructions of summer aridity for the Middle Atlas during the last 5000 years. Higher $\Delta^{13}\text{C}$ values indicate wetter conditions. [Color figure can be viewed at wileyonlinelibrary.com].

composition and site conditions, rather than a robust climate signal, which is only attained by isolating a specific species and tissue type, as evidenced here by the isolated *Cedrus* pollen.

Reconstructed Holocene aridity from isolated fossil *Cedrus* pollen

Our findings from isolated fossil *Cedrus* pollen reveal that carbon isotope discrimination has decreased in the Middle Atlas region, and hence summer aridity has increased during the last ~5000 years, indicating the climate has become drier. Around 5100 cal a BP, the climate was relatively humid compared to the present day, and similarly for around 8200 cal a BP, which has been described as a period of cool summers and dry winters (Campbell *et al.*, 2017). It is noteworthy also that fossil $\Delta^{13}\text{C}$ values are outside the range of the site averages for the present-day Middle Atlas and are similar to trees growing in the Rif mountains today (Bell *et al.*, 2017), further emphasizing a much more humid bioclimate than present in the Middle Atlas.

The widespread expansion of *Cedrus* forests in the Middle Atlas occurred before 5000 cal a BP (Fig. 6A), with maximum forest established between 5000 and 4400 BP at Lake Sidi Ali (Campbell *et al.*, 2017) and Col du Zad (Campbell, 2017). The gradual aridification of the area (Fig. 6C) appears to have promoted the long-term decline in forest cover during the late Holocene (4300 cal a BP to present). Conversely, the more humid summer conditions of the mid-Holocene and the period around 8200 cal a BP promoted the expansion of *Cedrus atlantica* in the Middle Atlas (Zielhofer *et al.*, 2019).

Changes in forest density, here indicated by the arboreal/non-arboreal pollen ratio, here indicated by the arboreal/non-arboreal pollen ratio (Magri, 1994), shows peak total forest occurred ~300 years after aridity started to increase (Fig. 6B), while *Cedrus* forest continued to expand for another ~300 years, stabilizing around 4300 BP before declining. This apparent lag may reflect the long-lived nature of the species (lifespan up to 1000 years) and its resilience to moisture stress, which is particularly true in older specimens (Linares *et al.*, 2013). Importantly, the comparison may indicate the lagged population response to climate change as reflected in pollen

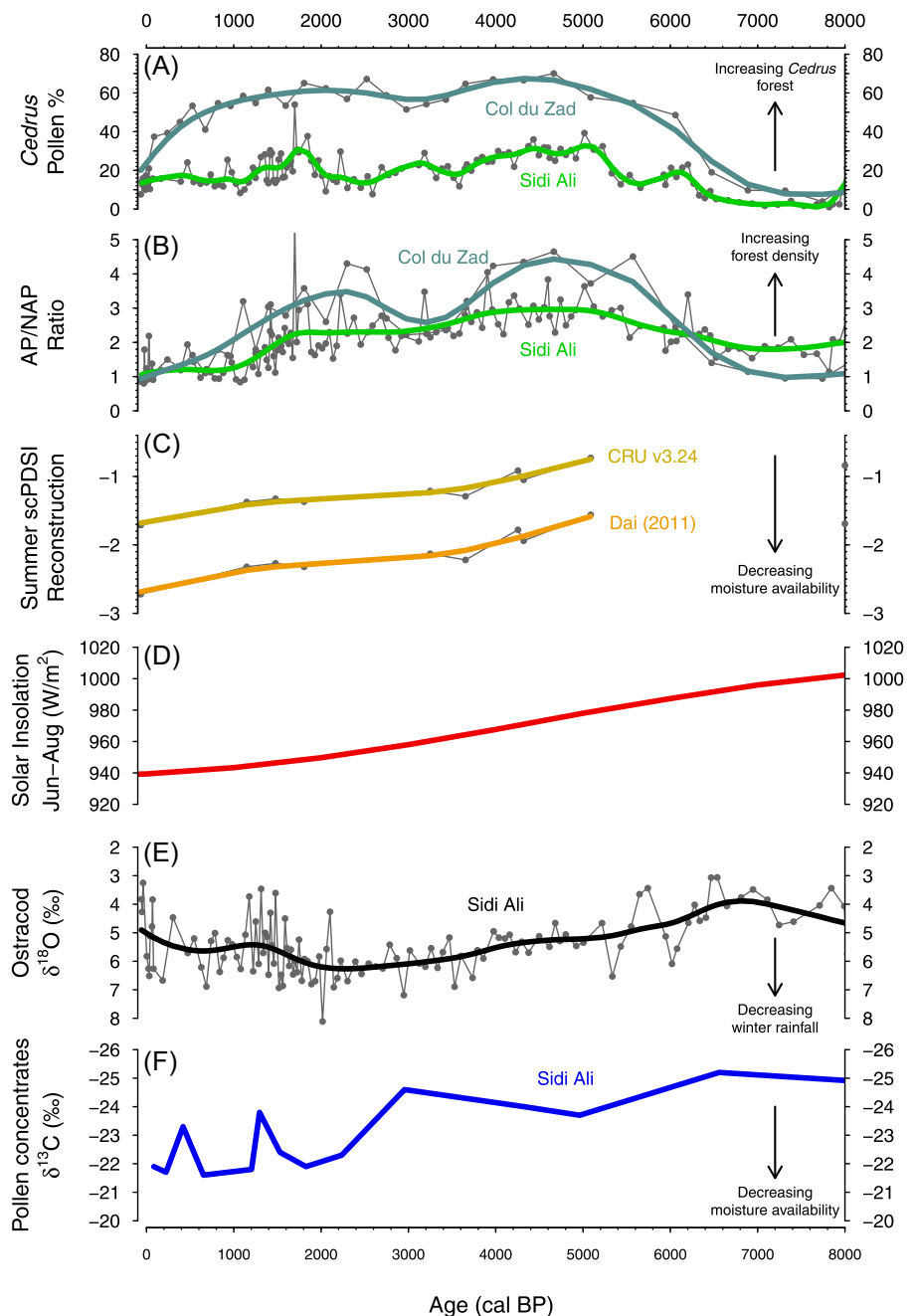


Figure 6. Comparison of different proxies. The pollen record at Lake Sidi Ali (Campbell *et al.*, 2017) and Col du Zad (Campbell, 2017) indicating an overall trend (thick green lines) of: (A) decreasing *Cedrus* pollen abundance, and (B) decreasing arboreal pollen abundance since the mid-Holocene. (C) Summer aridity reconstructed from fossil *Cedrus* pollen for the last ~5000 years (this study). (D) Summer solar insolation reconstruction from Berger and Loutre (1991). (E) $\delta^{18}\text{O}$ record from ostracods at Lake Sidi Ali (Zielhofer *et al.*, 2017a). (F) $\delta^{13}\text{C}$ of pollen concentrates from Fletcher *et al.* (2017). [Color figure can be viewed at wileyonlinelibrary.com].

assemblages versus a rapid response in isotopes. This lag effect may skew the interpretation of the timing of climate changes when based on pollen abundances alone. Our reconstruction falls within the reconstructed scPDSI values from tree-rings for the last ~900 years in Morocco (Esper *et al.*, 2007) and North Africa (Touchan *et al.*, 2011), suggesting that the extremes of long-term change over the Holocene may have been similar in amplitude to the extremes of high-frequency (annual, decadal and centennial) variability. To date, we do not have sufficient sampling resolution for the last 1000 years to test for the well-known dry to humid transition from the MCA to LIA as observed in tree-ring records (Esper *et al.*, 2007).

A complexity for the study of past hydrological change in semi-arid regions is disentangling the influence of precipitation and temperature on drought regime. The summer aridity signal recorded in the carbon isotope composition of *Cedrus* pollen (Fig. 6C) may effectively integrate precipitation and temperature influence on summer soil moisture availability. Since *C. atlantica* is a shallow-rooted species (Aussenac, 1984), it lacks access to deep soil moisture resulting from winter precipitation. The choice of scPDSI here, and in the tree-ring studies as the reconstruction target, reflects the need to integrate temperature and precipitation impacts on bioclimatic moisture availability. However, as the aridification trend parallels the reduction in summer insolation (Fig. 6D), it would appear that increasing temperature was not the primary driver. If precipitation remained high throughout the Late Holocene, then temperature would not have been a limiting factor on leaf stomatal conductance, because soil moisture reserves would allow evaporative demands to be met through high transpiration. As such, the summer aridification trend could not simply be explained by rising temperature alone, and it is likely that decreasing precipitation is the main driver of the aridity trend detected here. This interpretation draws support both from the dominant influence of precipitation on C3 plant $\delta^{13}\text{C}$ (Rao *et al.*, 2017) and leaf physiological responses in trees in semi-arid ecosystems (Grossiord *et al.*, 2017).

Our results also show a similar trend to the ostracod $\delta^{18}\text{O}$ record from Lake Sidi Ali (Zielhofer *et al.*, 2017a) (Fig. 6E), and $\delta^{13}\text{C}$ values of pollen concentrates from Lake Sidi Ali obtained during accelerator mass spectrometry ^{14}C dating (Fletcher *et al.*, 2017) (Fig. 6F), particularly between ~5000 and ~2000 cal a BP. The main driver of change in $\delta^{18}\text{O}$ over the Holocene is interpreted as variability in winter rainfall (Zielhofer *et al.*, 2017a), suggesting a coherent annual climate signal (coupled summer and winter drying) during this period with an imprint on the pollen concentrate $\delta^{13}\text{C}$. Subsequently, the apparent divergence between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ trends within the last ~2000 years may suggest an increase in seasonal contrast, with increasing winter rainfall, but continuing summer drying. However, the mixed nature of material in the pollen concentrates makes them less representative of a seasonal signal, and thus far the resolution of the isolated *Cedrus* pollen is insufficient to confirm a change in seasonality. Overall, the comparison highlights the potential to decouple different seasonal climate signals by integrating multiple isotope proxies.

Practicalities and wider implications

Each pollen sample in this study provides a robust picture of the average regional climate conditions for the represented time period. By increasing the sampling resolution, there is great potential to enhance the understanding of bioclimatic changes not only for long-term trends but also of Holocene rapid climate changes. It is envisaged that for future analysis, the number of grains required for isotope analysis could be

much smaller due to refinements to the technique and improvements in EA-IRMS sensitivities. Indeed, at 20 μg carbon, the number of fossil pollen required could be reduced by more than 1300 to ~2700 grains, while at 5 μg carbon (the theoretical minimum) the total number of grains required is only around 650. The equivalent picking time would then be approximately 2–3 h, which is comparable to the time it takes to analyse a slide for traditional pollen analysis. An alternative approach to analysing pollen $\delta^{13}\text{C}$ uses a spooling wire microcombustion device, which can analyse pollen samples using < 100 grains (Nelson *et al.*, 2008; Nelson, 2012; Griener *et al.*, 2013). However, this requires more specialized equipment, which is not as widely available as standard EA-IRMS setups.

More widely, our approach has exciting potential for new applications in other Mediterranean or global drought-stressed environments. This pollen-based isotopic aridity proxy can help to overcome uncertainties in the interpretation of vegetation records as to the drivers of change in pollen assemblages – a traditional challenge facing palaeoecological interpretation of the Late Holocene in the Mediterranean region (Jalut *et al.*, 2009; Mercuri *et al.*, 2011; Roberts *et al.*, 2011). It may also help to disentangle the vegetation–climate disequilibrium (where vegetation composition may lag climate change) by comparison of the climatic signal from the stable isotope analysis, and the vegetation reconstruction from the pollen assemblage – in the same samples and without chronological uncertainty. Furthermore, because pollen-based isotope reconstructions can be carried out over longer time periods than is possible with tree-ring studies, this technique can offer new insights into the dynamics of long-term climate changes. As yet, we do not know the prevailing summer aridity conditions during the Early Holocene, but we hypothesize dry conditions which favoured the expansion of deep-rooting sclerophyll trees (Campbell *et al.*, 2017). To date, our findings indicate a Mid- to Late Holocene aridification trend in north-west Africa, as found in the wider western Mediterranean region (Magny *et al.*, 2002; Carrión *et al.*, 2010; Roberts *et al.*, 2011), and adjacent Sahara (Zielhofer *et al.*, 2017b).

Conclusions

Our study offers new insights into climate change during the Late Holocene and documents the first use of stable carbon isotope analysis on fossil *Cedrus* pollen. Our study uses a new method to concentrate and isolate fossil *Cedrus* pollen from pollen assemblages that does not use traditional carbon-bearing chemicals that would otherwise contaminate the isotope signal. Our findings indicate that summer aridity in the Middle Atlas has increased during the last 5000 years. The trend corresponds with an overall decline in arboreal pollen and *Cedrus* pollen, supporting a role for moisture stress as a key driver of regional Late Holocene vegetation change. Our findings also provide a tentative suggestion of centennial-scale disequilibria between total forest development and climate change.

The aridity trend corresponds to the decline in incoming summer solar insolation, and we suggest that the aridity trend was driven mainly by decreased summer precipitation, rather than increasing temperatures. Although thus far our results do not reveal short-term climate events as observed in the tree-ring records, the overall range of reconstructed scPDSI values is comparable. Increasing the resolution of pollen-based isotope proxies would offer the same short-term insights provided by tree-ring records, but over longer periods. Overall, our findings help to confirm a summer aridification trend in north-west Africa and the wider western Mediterranean region

since ~5000 cal a BP and underline the value of further application of stable isotope research to fossil pollen.

Supporting information

Additional supporting information can be found in the online version of this article.

Fossil *Cedrus* pollen isotope results and corrections applied. Modern *Cedrus atlantica* pollen normalized isotope results. *Cedrus* pollen data for Lake Sidi Ali and Col du Zad. Pollen concentrates and bulk sediment isotope results.

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Abbreviations. EA-IRMS, elemental analysis isotope ratio mass spectrometry; IPE, International Plant Exchange; ISE, International Soil Exchange; LIA, Little Ice Age; MCA, Medieval Climate Anomaly; scPDSI, self-calibrating Palmer drought severity index; SPT, sodium polytungstate.

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