



# Stable carbon isotope analysis of *Cedrus atlantica* pollen as an indicator of moisture availability



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## ABSTRACT

Stable carbon isotope analysis of pollen provides potential for reconstruction of past moisture availability in the environment on longer time-scales compared to isotope analysis of plant tissue. Here we show that the carbon isotopic compositions ( $\delta^{13}\text{C}$ ) of pollen, sporopollenin, leaf and stem tissues of *Cedrus atlantica* are strongly related. Untreated pollen  $\delta^{13}\text{C}$  has a significant linear relationship with sporopollenin  $\delta^{13}\text{C}$  ( $r^2 = 0.97$ ,  $p < 0.0001$ ) which is relatively depleted in  $^{13}\text{C}$  by an average 1.5‰. Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) by sporopollenin (derived from pollen  $\delta^{13}\text{C}$  values) is related to mean annual ( $r^2 = 0.54$ ,  $p < 0.001$ ) and summer precipitation ( $r^2 = 0.63$ ,  $p < 0.0001$ ). A 100 mm increase in mean annual precipitation results in sporopollenin  $\Delta^{13}\text{C}$  increasing by 0.52‰, or by 1.4‰ per 100 mm summer precipitation. There is a stronger relationship between sporopollenin  $\Delta^{13}\text{C}$  and long-term annual scPDSI ( $r^2 = 0.86$ ,  $p < 0.0001$ ) and summer scPDSI ( $r^2 = 0.86$ ,  $p < 0.001$ ) aridity indexes, with reduced  $\Delta^{13}\text{C}$  as aridity increases. These relationships suggest that stable carbon isotope analysis of *C. atlantica* fossil pollen could be used as a quantitative proxy for the reconstruction of summer moisture availability in Northwest Africa.

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## 1. Introduction

The stable carbon isotope composition ( $\delta^{13}\text{C}$ ) of plant tissue is well understood as discrimination against  $^{13}\text{C}$  that occurs during photosynthesis (Park and Epstein, 1960; O'Leary, 1981; Farquhar et al., 1989). It can be used for a range of applications including determining photosynthetic pathways (Tieszen et al., 1979; Farquhar, 1983; O'Leary, 1988), inferring plant physiological differences (Francey et al., 1985; Körner et al., 1991), water-use efficiency (Farquhar and Richards, 1984; Marshall and Zhang, 1994; Warren et al., 2001) and for reconstruction of atmospheric and climate conditions of past and present environments (Feng and Epstein, 1995; Dawson et al., 2002).

Pollen is an ideal material for environmental and climate reconstructions as it is composed primarily of a highly resistant biopolymer *sporopollenin* (Havina, 1967) which can be preserved in geological archives for millions of years (Traverse, 2007). Although the exact chemical composition is unknown, sporopollenin contains aliphatic and phenolic components, the latter largely comprising p-coumaric acid and ferulic acid (Bergen et al., 2004; Blokker et al., 2006). Its chemical structure remains relatively stable over time unless subjected to extreme conditions

(Fraser et al., 2014), thereby retaining information relevant to environmental conditions during its development period.

Previous studies on pollen stable carbon isotope composition have shown that the  $\delta^{13}\text{C}$  value of Poaceae pollen reflects C3 and C4 photosynthetic pathways (Amundson et al., 1997; Descolas-Gros et al., 2001; Nelson et al., 2006, 2007), useful for understanding changes in C3 to C4 species abundance in grassland environments which typically differ in their response to environmental controls (Nelson et al., 2008). Loader and Hemming (2004), found correlations between climate conditions and stable isotope composition of *Pinus sylvestris* L. pollen during its development period. Jahren (2004) analysed  $\delta^{13}\text{C}$  values of pollen, leaf, and stem tissue of 175 different plant species, and found pollen  $\delta^{13}\text{C}$  to be highly correlated with the  $\delta^{13}\text{C}$  of parent leaf material, suggesting that the  $\delta^{13}\text{C}$  of bulk pollen could infer  $\delta^{13}\text{C}$  of past plant communities to within 1.5‰. The isotopic composition of sporopollenin can also be derived from untreated pollen as the two are strongly related. For example, Nelson (2012) found significant relationships between the two in several *Ambrosia* and *Artemisia* species ( $r^2 = 0.85$ – $0.99$ ). Discrimination against  $^{13}\text{C}$  in sporopollenin was also shown to strongly correlate with the Palmer Drought Severity Index (PDSI), suggesting it to be a good indicator of moisture stress (Nelson, 2012). Similarly, Griener et al. (2013) evaluated the relationship between the carbon isotopic composition of modern *Nothofagus* sporopollenin and precipitation, finding the two to be strongly related. A

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reconstruction of moisture availability using fossil *Nothofagidites* sporopollenin suggested moisture availability declined during the Late Eocene on the Antarctic Peninsula.

Overall, the carbon isotopic composition of plant tissue has been shown to be related to moisture availability (Farquhar et al., 1989; Warren et al., 2001; Dawson et al., 2002; Ferrio and Voltas, 2005; Diefendorf et al., 2010). However, species responses can vary significantly (Fardusi et al., 2016). As pollen is more widely preserved in geological archives compared to leaf tissue, the same relationship occurring in pollen should allow longer time-scale reconstruction of past moisture availability. Further characterisation of this relationship for individual species is needed to understand specific species response (Loader and Hemming, 2004), particularly for species in semi-arid areas where drought impacts on plants are significant (Ferrio et al., 2005).

The Atlas cedar; *Cedrus atlantica* (Endl.) Manetti ex Carrière is a montane conifer found in semi-arid and humid areas of Morocco and Algeria, typically occurring on exposed north and north western slopes of the Rif and Atlas (Middle, High, and Algerian Tell) mountain ranges at an elevation of 1400–2600 m (Benabid, 1982; Farjon, 1990). It is cold tolerant and drought resistant, but sensitive to extreme or prolonged drought stress (Aussenac, 1984; Cheddadi et al., 2009; Linares et al., 2011, 2013; Rhanem, 2011; Ilmen et al., 2014). In the last century, more than 75% of original *C. atlantica* forest has been lost due to climate and anthropogenic impacts (Benabid and Fennane, 1994), with evergreen holm oak (*Quercus rotundifolia* Lam., syn. *Quercus ilex* var. *rotundifolia* (Lam) Trab.) flourishing in its place in many areas. Morocco is a crucial area for the reconstruction of climate in North Africa (Rogon, 1987; Lamb et al., 1989). The region is sensitive to the effects of climate change, with future predictions of increased temperatures and reduced precipitation (Giorgi, 2006; Born et al., 2008; Solomon et al., 2009).

Fossil pollen records suggest *Cedrus* was present across parts of southern Europe, particularly widespread in Italy during the early Pleistocene (Magri et al., 2017). However, the European record is not conclusive as many of these early occurrences comprise only discontinuous single grains, present especially from the Middle Pleistocene (Magri et al., 2017), which may not indicate local presence and instead result from long-distance transport from North Africa (Magri and Parra, 2002). The disappearance of *Cedrus* from Europe nevertheless appears linked to profound shifts in climate throughout the Quaternary (Magri, 2012). Although there is little information from Northwest Africa regarding Pleistocene history of *Cedrus atlantica*, genetic studies and pollen records suggest it was present through the Last Glacial Maximum in coastal refugia locations, while its current distribution across the Middle Atlas was established since 10,000 years BP (Terrab et al., 2008). The trends suggest it is vulnerable to changing climate conditions (Magri, 2012), but can adapt and survive in new areas with more favourable climate. As a moisture sensitive tree, it should be an ideal candidate for testing  $\delta^{13}\text{C}$  against water stress, while the summer pollen development season for this autumn pollinating species offers potential for the reconstruction of summer climate conditions.

Here we present data showing the carbon isotopic composition of modern *Cedrus atlantica* pollen, sporopollenin, leaf and stem tissue from samples across the core biogeographical range in the Middle Atlas, Morocco and from areas outside the native range. We aim to (1) determine the range and variability of  $\delta^{13}\text{C}$  of *C. atlantica* pollen and its relationship with sporopollenin, leaf, and stem  $\delta^{13}\text{C}$ ; and (2) determine whether the carbon isotopic composition of pollen/sporopollenin of *C. atlantica* is a reliable indicator of moisture availability, and thus could be used as a long-term palaeoenvironmental proxy.

## 2. Materials and methods

### 2.1. Sample collection and preparation

Modern pollen and tissue samples were collected in late September 2015 from *Cedrus atlantica* trees in the Middle Atlas ( $n = 72$ ) and Rif

mountains ( $n = 2$ ) (Fig. 1). Sample locations in the Middle Atlas were chosen to cover a variety of environments at different altitudes along a NW to SE gradient. Sampling of individual trees was effectively opportunistic as pollinating specimens were rare and widely interspersed in these areas. Additional samples ( $n = 19$ ) were obtained from non-native specimens from Europe and USA in September and October 2014–2015. Typical sample location characteristics and botanical gardens are listed in Table 1, with individual sample details in Table 2 and Table 3. Detailed information on individual tree specimens (including slope angle/aspect, diameter at breast height (DBH), tree height, density of surrounding trees) is available in supplementary material 1. For each sample, multiple ( $> 10$ ) strobili and tissue materials were collected from a single specimen. *Cedrus atlantica* strobili typically only develop on a small number of exposed branches rather than the entire tree. For consistency, leaf samples were taken from the same branches as the strobili, and stem were taken from the stem growing directly underneath the strobili. Samples were washed in deionised water, freeze-dried and stored in glass vials at 4 °C.

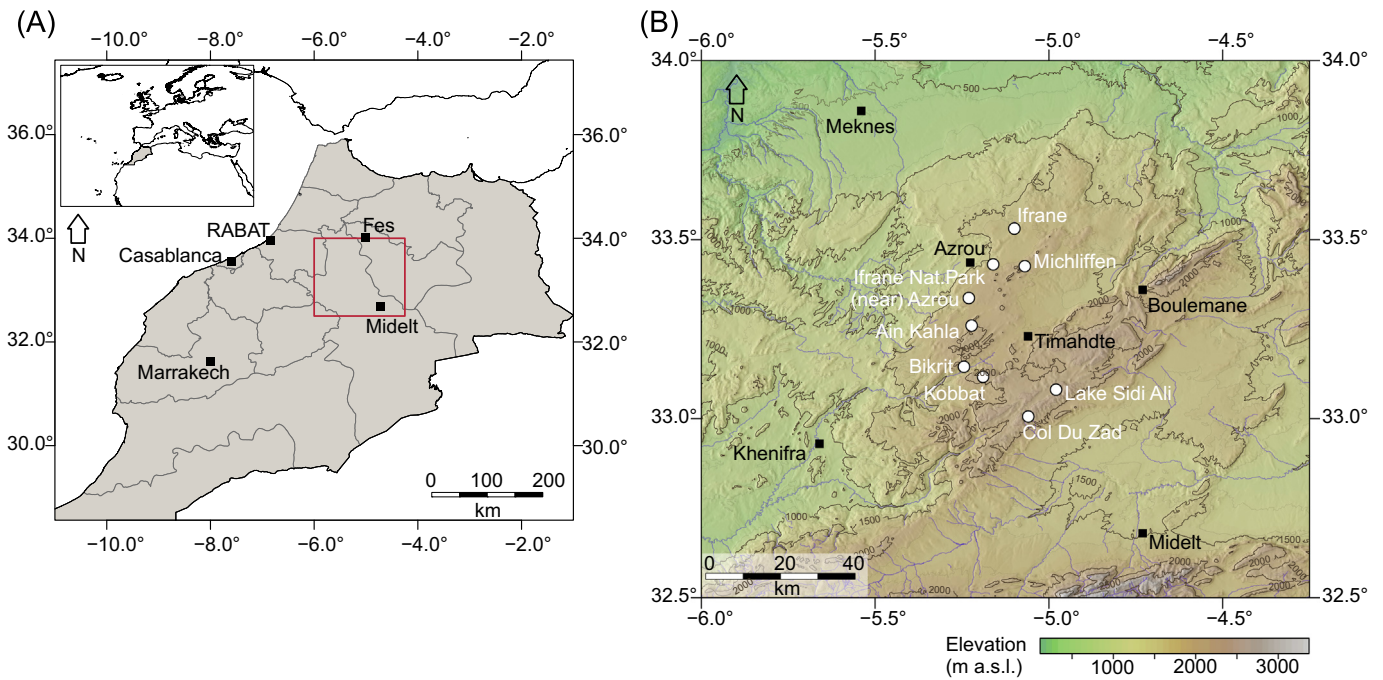
Pollen grains were extracted by shaking strobili in sieves of 100  $\mu\text{m}$  and 30  $\mu\text{m}$  with material retained in-between, and any contamination was removed by visual inspection. Sporopollenin was isolated from untreated pollen following Loader and Hemming (2000). Untreated pollen was placed in high purity concentrated  $\text{H}_2\text{SO}_4$  for 45 min, then treated by repeated washes in class 1 deionised water, centrifuging at 3000 rpm for 5 min until filtrate ran clear (typically eight or more washes). Differential staining techniques using Safranin and Fast Green (Loader and Hemming, 2000) were used to check the purity of the extracted sporopollenin. Whole leaf and whole stem material was homogenised using a mortar and pestle for analysis.

### 2.2. Stable isotope analysis

Stable carbon isotope analysis was carried out by the Natural Environment Research Council Life Sciences Mass Spectrometry Facility at CEH Lancaster on all 93 untreated pollen samples, with additional analysis on a subset of 30 samples of sporopollenin, leaf, and stem tissue. Samples were dried at 105 °C for 1 h and cooled in a desiccator. A high precision microbalance (Sartorius Ltd.) was used to weigh 0.32 mg (untreated pollen), or 0.40 mg (sporopollenin, leaf, and stem) of material for each sample, which was placed in a 6 × 4 mm tin capsule. Capsules were combusted using an automated Eurovector elemental analyser coupled to an Isoprime Isotope Ratio Mass-Spectrometer (Isoprime Ltd.). An in-house working standard of vegetation (plain flour) was analysed after every twelfth sample resulting in an analytical precision of 0.09‰. Data were normalised by single point anchoring, with the in-house standard calibrated against the National Institute of Standards and Technology (NIST) certified reference material; NIST no. 8542 (Sucrose-ANU). Results are expressed in delta notation:  $\delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$  (‰) where R is the ratio of  $^{13}\text{C}$  to  $^{12}\text{C}$  in the analysed sample and standard accordingly.  $\delta^{13}\text{C}$  results are expressed relative to the international standard Vienna Pee Dee Belemnite (VPDB). Negative  $\delta^{13}\text{C}$  values indicate the sample is depleted in the heavy isotope ( $^{13}\text{C}$ ) relative to this standard.

### 2.3. Data processing and analysis

For plant tissue, where the source of carbon is atmospheric  $\text{CO}_2$ , isotope discrimination ( $\Delta$ ) is often used to convey the degree to which plant tissue “avoids” the uptake of the heavy isotope during photosynthesis (Michener and Lajtha, 2007). Therefore, greater discrimination (more positive  $\Delta^{13}\text{C}$  values) is equivalent to more negative  $\delta^{13}\text{C}$  values in plant tissue. As the  $\delta^{13}\text{C}$  value of atmospheric  $\text{CO}_2$  has changed throughout time, indicated by ice core records (Francey et al., 1999; Lourdantou et al., 2010), it is necessary to account for the effects of these changes on plant tissue  $\delta^{13}\text{C}$  (Schubert and Jahren, 2015) in climate reconstructions. Therefore, we report the  $\delta^{13}\text{C}$  values, but



**Fig. 1.** (A) Map of Morocco and study area (red box). (B) Middle Atlas region topographic map (U.S. Geological Survey, 2017), with sampling locations (white circles), and towns (black squares) indicated.

additionally calculate the  $\Delta^{13}\text{C}$  value for analysis of climate relationships. Pollen  $\delta^{13}\text{C}$  values were converted to  $\Delta^{13}\text{C}$  using the formula:  $\Delta^{13}\text{C} (\text{‰}) = (\delta_A - \delta_B) / (1 + \delta_B \div 1000)$  where  $\delta_A$  is the  $\delta^{13}\text{C}$  of atmospheric  $\text{CO}_2$ , and  $\delta_B$  is the  $\delta^{13}\text{C}$  of the sample (Farquhar et al., 1989). A consistent atmospheric  $\text{CO}_2$   $\delta^{13}\text{C}$  value of  $-8.34\text{‰}$  was obtained from NOAA Earth System Research Laboratory (ESRL) South Pole station (White et al., 2015).

Climate data for all sample locations were extracted from CRU TS v3.24.01 high resolution ( $0.5^\circ$ ) gridded datasets (Harris et al., 2014). Aridity data was also extracted from the self-calibrating Palmer Drought Severity Index (scPDSI) (Dai, 2011), and an aridity index ( $\text{AI}_U$ ) was calculated ( $P/\text{PET}$ ) using CRU TS v3.24.01 data. All extracted climate data was averaged over a 30-year period (1986–2015). While these datasets are useful across the entire range of sample locations, they do not fully capture the highly variable precipitation regime in the Middle Atlas, resulting from a strong NW to SE gradient of precipitation reduction

associated with prevailing air masses and Föhn effects related to SW to NE topographic ridges. For the Middle Atlas region, we also performed an Inverse Distance Weighted (IDW) interpolation based on mean annual precipitation for 19 stations with data obtained from MarocMeteo, National Oceanic and Atmospheric Administration (NOAA, 2017), and several publications (Jihad, 2003; Sayad et al., 2011; Daki et al., 2015), available in supplementary material 2. Although the date ranges of the data for these stations vary (between 1970 and 2015), which may affect the interpolation (due to high inter-annual precipitation variability), the resulting interpolation (Fig. 2) is comparable to other published results such as in Fink et al. (2010).

Middle Atlas elevation profile was created using Profile Tool plugin for QGIS (QGIS Development Team, 2017), based on Global Multi-resolution Terrain Elevation Data 2010 (GMTED2010) (U.S. Geological Survey, 2017). All data and statistical analyses were carried out using R (R Core Team, 2016).

**Table 1**  
Sample location, environmental notes and typical climate conditions.

Site code <sup>a</sup>	Location	Environment	Mean annual precipitation (mm) <sup>b</sup>	Temperature range ( $^\circ\text{C}$ ) <sup>c</sup>
MA_IFR	Ifrane	Park (irrigation)	474 (836)	−1.8 to 28.0
MA_MIC	Michliffen	Mixed open forest at ski resort	516 (714)	−3.0 to 28.5
MA_INP	Ifrane National Park	Mostly cedar forest (protected from human impact)	516 (747)	−1.7 to 29.8
MA_COL	Col Du Zad	Sparse open forest, close to road.	470 (515)	−3.8 to 29.6
MA_ALI	Lake Sidi Ali	Mixed open forest close to lake (recent efforts to protect from human impact)	470 (408)	−3.3 to 29.2
MA_BIK	Bikrit	Mixed cedar/oak forest (animal grazing)	516 (738)	−1.0 to 30.5
MA_KOB	Kobbat (near Bikrit)	Mixed open forest (animal grazing)	516 (667)	−3.8 to 27.7
MA_KAH	Ain Kahla	Mixed cedar/oak forest (animal grazing)	516 (695)	−3.0 to 28.5
MA_AZR	(near) Azrou	Mixed cedar/oak forest (animal grazing)	516 (696)	−2.2 to 29.3
MA_RIF	Rif mountains	Mixed forest	654	−1.5 to 22.3
UK_WB	Westonbirt, UK	Westonbirt Arboretum	854	0.9 to 20.7
UK_MAN	Manchester, UK	Urban greenspaces	890	1.4 to 19.6
US_BOS	Boston, USA	Arnold Arboretum, Harvard University	1236	−9.2 to 27.1
FR_PAR	Paris, France	Paris Botanical Gardens	618	1.8 to 24.8
FR_BOR	Bordeaux, France	Urban park	958	2.4 to 25.2
ES_PYR	Jaca, Pyrenees, Spain	Urban, trees planted along roads	753	−1.9 to 26.0

<sup>a</sup> Codes starting MA\_ are located in Morocco.

<sup>b</sup> CRU data averaged over 30 years (1986–2015). Values in parentheses indicate interpolated precipitation values (Fig. 2).

<sup>c</sup> Mean annual minimums and maximums.

**Table 2**

Results of stable carbon isotope analysis on untreated pollen, sporopollenin, leaf, and stem tissue for each sample (n = 30).

Sample	Longitude	Latitude	Altitude	Collection date	Pollen $\delta^{13}\text{C}$ (‰)	Sporopollenin $\delta^{13}\text{C}$ (‰)	Leaf $\delta^{13}\text{C}$ (‰)	Stem $\delta^{13}\text{C}$ (‰)
MA_MIC_03	-5.0759	33.4123	1906	23/09/2015	-24.9	-26.8	-27.1	-25.3
MA_MIC_08	-5.0774	33.4088	1947	23/09/2015	-23.0	-24.6	-24.9	-24.4
MA_MIC_12	-5.0828	33.4138	1973	23/09/2015	-23.6	-25.7	-25.5	-25.1
MA_MIC_13	-5.0810	33.4149	1939	23/09/2015	-24.1	-25.7	-25.9	-25.9
MA_MIC_14	-5.0803	33.4152	1954	23/09/2015	-25.2	-26.9	-27.4	-27.0
MA_MIC_15	-5.0800	33.4154	1939	23/09/2015	-23.3	-24.9	-26.8	-25.4
MA_MIC_16	-5.0705	33.4228	1931	23/09/2015	-23.4	-25.2	-25.7	-24.7
MA_INP_18	-5.1722	33.4202	1707	24/09/2015	-24.8	-26.2	-27.1	-26.0
MA_INP_20	-5.1711	33.4207	1714	24/09/2015	-24.8	-26.8	-26.2	-25.9
MA_INP_21	-5.1710	33.4196	1730	24/09/2015	-26.6	-28.1	-27.3	-26.7
MA_INP_23	-5.1724	33.4193	1732	24/09/2015	-27.6	-29.2	-28.6	-27.6
MA_COL_25	-5.0706	32.9865	2080	24/09/2015	-23.6	-25.1	-25.9	-24.7
MA_COL_29	-5.0736	32.9952	2127	24/09/2015	-23.5	-25.1	-25.0	-24.2
MA_COL_30	-5.0735	32.9955	2129	24/09/2015	-24.3	-26.0	-26.9	-25.6
MA_ALL_33	-4.9926	33.0701	2122	24/09/2015	-24.3	-25.7	-25.6	-24.8
MA_ALL_34	-4.9923	33.0632	2124	24/09/2015	-23.7	-25.1	-25.0	-24.9
MA_ALL_35	-4.9910	33.0708	2143	24/09/2015	-23.5	-25.5	-26.1	-25.5
MA_ALL_37	-4.9905	33.0705	2162	24/09/2015	-24.5	-25.9	-25.3	-25.3
MA_ALL_38	-4.9914	33.0696	2174	24/09/2015	-23.2	-24.8	-24.8	-24.6
MA_ALL_39	-4.9914	33.0693	2194	24/09/2015	-23.1	-24.6	-24.9	-24.7
MA_ALL_40	-4.9909	33.0688	2216	24/09/2015	-23.9	-24.8	-25.9	-24.9
MA_KOB_50	-5.2047	33.1072	2094	27/09/2015	-25.1	-26.4	-27.0	-25.8
MA_KOB_51	-5.2049	33.1068	2073	27/09/2015	-25.5	-26.6	-27.1	-26.1
MA_KOB_53	-5.2057	33.1055	2078	27/09/2015	-24.5	-25.8	-26.9	-25.9
MA_KOB_55	-5.2053	33.1052	2069	27/09/2015	-23.3	-24.9	-25.5	-24.2
UK_WB_01	-2.2331	51.6029	124	09/10/2014	-28.6	-29.2	-30.0	-29.2
UK_WB_03	-2.2117	51.6081	124	09/10/2014	-27.7	-28.9	-28.7	-28.3
UK_WB_06	-2.2075	51.6116	140	09/10/2014	-28.9	-30.8	-28.9	-27.5
UK_MAN_07	-2.1894	53.4175	68	30/09/2015	-30.0	-31.8	-30.7	-29.6
ES_PYR_02	-0.5526	42.5662	820	10/10/2015	-25.1	-26.7	-26.1	-25.2
				Average	-24.9	-26.4	-26.6	-25.8
				Min	-30.0	-31.8	-30.7	-29.6
				Max	-23.0	-24.6	-24.8	-24.2
				Range	-7.0	-7.3	-5.9	-5.4
				Standard deviation	1.89	1.86	1.52	1.40

### 3. Results

#### 3.1. Untreated pollen, sporopollenin, leaf and stem $\delta^{13}\text{C}$ comparison

A comparison of untreated pollen, sporopollenin, leaf and stem tissue (Table 2) shows *Cedrus atlantica* untreated pollen is more enriched in  $^{13}\text{C}$  relative to sporopollenin, leaf, and stem in all samples except one (UK\_WB\_06). Untreated pollen  $\delta^{13}\text{C}$  values are on average 1.5‰ less negative than sporopollenin, 1.7‰ than leaf and 0.9‰ than stem. The smallest differences in  $\delta^{13}\text{C}$  values are observed between sporopollenin and leaf, with sporopollenin  $\delta^{13}\text{C}$  averaging 0.2‰ less negative values; a slightly larger difference is observed between sporopollenin and stem, with stem averaging 0.7‰ more negative values than sporopollenin  $\delta^{13}\text{C}$  (Fig. 3).

Regression analysis shows there is a significant relationship between untreated pollen and sporopollenin  $\delta^{13}\text{C}$  ( $r^2 = 0.97$ ,  $p < 0.0001$ ) (Fig. 3A), untreated pollen and leaf ( $r^2 = 0.87$ ,  $p < 0.0001$ ) and untreated pollen and stem ( $r^2 = 0.90$ ,  $p < 0.0001$ ) (Fig. 3C). Sporopollenin also shows a significant relationship with leaf ( $r^2 = 0.84$ ,  $p < 0.0001$ ), and stem ( $r^2 = 0.84$ ,  $p < 0.0001$ ) (Fig. 3B).

#### 3.2. Pollen isotopic composition

Isotopic analysis of 93 untreated pollen samples (Table 3, Fig. 4) reveals an average  $\delta^{13}\text{C}$  of -24.5‰ for Moroccan samples compared to -27.3‰ for non-Moroccan samples. For all samples the average  $\delta^{13}\text{C}$  was -25.1‰, ranging from -23‰ to -30‰. Sporopollenin  $\delta^{13}\text{C}$  was modelled for all samples based on the regression model in Fig. 3A, with an average  $\delta^{13}\text{C}$  of -26.6‰, and ranging from -24.6‰ to -31.4‰. Variability of isotopic composition of untreated pollen by sample location (Fig. 4) shows an average intra-site variability of  $\delta^{13}\text{C}$  1.8‰, ranging from 0.6‰ up to 3.8‰.

Correlations between  $\delta^{13}\text{C}$  of untreated pollen and micro-scale environmental variables and specimen characteristics for those samples collected in the Middle Atlas (Fig. 5), show that slope angle and the number of trees close to the sampled tree are positively correlated with  $\delta^{13}\text{C}$ , i.e. greater slope angles and higher density of surrounding trees are associated with less negative  $\delta^{13}\text{C}$ . Slope aspect shows only minor positive correlation, although the majority of trees were sampled from N and NW facing slopes. The sampled tree's DBH and tree height both have a negative correlation with  $\delta^{13}\text{C}$ , i.e. larger and taller trees are associated with more negative  $\delta^{13}\text{C}$ .

#### 3.3. Isotope discrimination and moisture availability

With a view to palaeoclimatic reconstructions we compare sporopollenin  $\Delta^{13}\text{C}$  derived from the untreated pollen  $\delta^{13}\text{C}$  values (Table 3) to precipitation and aridity (as described in Section 2.3).  $\Delta^{13}\text{C}$  values were also averaged by their respective sample location given that fossil *Cedrus atlantica* pollen assemblages from terrestrial and lake sediments will comprise pollen originating from a number of different trees in the surrounding locality (Bell and Fletcher, 2016). The average isotope discrimination ( $\Delta^{13}\text{C}$ ) for untreated pollen across all samples was 17.2‰, ranging from 15.0‰ to 22.3‰. For sporopollenin, average  $\Delta^{13}\text{C}$  was 18.8‰ for all samples, ranging from 16.7‰ to 23.8‰ (Table 3).

Across the entire sample gradient, there is a significant relationship between sporopollenin  $\Delta^{13}\text{C}$  and mean annual precipitation ( $r^2 = 0.54$ ,  $p < 0.001$ ) (Fig. 6A), and a stronger relationship with summer season (June, July, August) precipitation ( $r^2 = 0.63$ ,  $p < 0.0001$ ) (Fig. 6B) using CRU gridded data. Based on the regression models, for every 100 mm increase in mean annual precipitation, sporopollenin  $\Delta^{13}\text{C}$  increases by 0.52‰; for summer precipitation,  $\Delta^{13}\text{C}$  increases by 1.4‰. With aridity, there is a strong significant relationship between sporopollenin  $\Delta^{13}\text{C}$  and annual scPDSI ( $r^2 = 0.86$ ,  $p < 0.0001$ ) (Fig. 6C), and

**Table 3**  
Results of stable carbon isotope analysis on untreated pollen samples (n = 93), and calculated isotope discrimination ( $\Delta^{13}\text{C}$ ) values.

Sample	Longitude	Latitude	Altitude	Collection date	Pollen $\delta^{13}\text{C}$ (‰)	Pollen $\Delta^{13}\text{C}$ (‰) <sup>a</sup>	Modelled sporopollenin $\delta^{13}\text{C}$ (‰) <sup>b</sup>	Modelled sporopollenin $\Delta^{13}\text{C}$ (‰) <sup>a</sup>
MA_IFR_01	-5.1055	33.5300	1654	23/09/2015	-26.2	18.3	-27.7	19.9
MA_IFR_02	-5.1054	33.5304	1652	23/09/2015	-25.4	17.5	-26.9	19.1
MA_MIC_03	-5.0759	33.4123	1906	23/09/2015	-24.9	17.0	-26.4	18.6
MA_MIC_04	-5.0756	33.4124	1923	23/09/2015	-24.3	16.3	-25.8	17.9
MA_MIC_05	-5.0772	33.4098	1911	23/09/2015	-26.6	18.8	-28.1	20.4
MA_MIC_06	-5.0776	33.4095	1921	23/09/2015	-25.0	17.1	-26.5	18.7
MA_MIC_07	-5.0778	33.4087	1935	23/09/2015	-25.2	17.3	-26.8	18.9
MA_MIC_08	-5.0774	33.4088	1947	23/09/2015	-23.0	15.0	-24.6	16.7
MA_MIC_09	-5.0798	33.4098	1947	23/09/2015	-23.2	15.2	-24.7	16.8
MA_MIC_10	-5.0809	33.4103	1975	23/09/2015	-23.4	15.4	-25.0	17.1
MA_MIC_11	-5.0805	33.4093	1974	23/09/2015	-23.1	15.1	-24.7	16.7
MA_MIC_12	-5.0828	33.4138	1973	23/09/2015	-23.6	15.6	-25.2	17.3
MA_MIC_13	-5.0810	33.4148	1939	23/09/2015	-24.1	16.2	-25.7	17.8
MA_MIC_14	-5.0803	33.4152	1954	23/09/2015	-25.2	17.3	-26.8	18.9
MA_MIC_15	-5.0799	33.4154	1939	23/09/2015	-23.3	15.3	-24.9	17.0
MA_MIC_16	-5.0705	33.4228	1931	23/09/2015	-23.4	15.4	-24.9	17.0
MA_MIC_17	-5.0680	33.4222	1927	23/09/2015	-25.2	17.3	-26.8	18.9
MA_INP_18	-5.1722	33.4202	1707	24/09/2015	-24.8	16.8	-26.3	18.4
MA_INP_19	-5.1738	33.4222	1722	24/09/2015	-23.7	15.8	-25.3	17.4
MA_INP_20	-5.1711	33.4207	1714	24/09/2015	-24.8	16.9	-26.3	18.5
MA_INP_21	-5.1710	33.4196	1730	24/09/2015	-26.6	18.8	-28.1	20.3
MA_INP_22	-5.1718	33.4185	1733	24/09/2015	-25.9	18.0	-27.4	19.5
MA_INP_23	-5.1724	33.4193	1732	24/09/2015	-27.6	19.8	-29.0	21.3
MA_COL_24	-5.0703	32.9864	2092	24/09/2015	-23.7	15.7	-25.2	17.3
MA_COL_25	-5.0706	32.9865	2080	24/09/2015	-23.6	15.6	-25.1	17.2
MA_COL_26	-5.0715	32.9863	2099	24/09/2015	-25.6	17.7	-27.1	19.3
MA_COL_27	-5.0710	32.9865	2093	24/09/2015	-24.5	16.6	-26.1	18.2
MA_COL_28	-5.0722	32.9949	2125	24/09/2015	-23.9	15.9	-25.4	17.5
MA_COL_29	-5.0736	32.9952	2127	24/09/2015	-23.5	15.5	-25.1	17.1
MA_COL_30	-5.0735	32.9955	2129	24/09/2015	-24.3	16.4	-25.9	18.0
MA_ALI_31	-4.9933	33.0707	2098	25/09/2015	-24.6	16.6	-26.1	18.2
MA_ALI_32	-4.9930	33.0701	2115	25/09/2015	-24.8	16.9	-26.4	18.5
MA_ALI_33	-4.9926	33.0701	2122	25/09/2015	-24.3	16.4	-25.9	18.0
MA_ALI_34	-4.9923	33.0632	2124	25/09/2015	-23.7	15.8	-25.3	17.4
MA_ALI_35	-4.9910	33.0708	2143	25/09/2015	-23.5	15.5	-25.1	17.2
MA_ALI_36	-4.9904	33.0707	2156	25/09/2015	-24.3	16.3	-25.8	17.9
MA_ALI_37	-4.9905	33.0705	2162	25/09/2015	-24.5	16.5	-26.0	18.1
MA_ALI_38	-4.9914	33.0696	2174	25/09/2015	-23.2	15.2	-24.7	16.8
MA_ALI_39	-4.9914	33.0693	2194	25/09/2015	-23.1	15.1	-24.7	16.7
MA_ALI_40	-4.9909	33.0688	2216	25/09/2015	-23.9	15.9	-25.5	17.6
MA_BIK_41	-5.2542	33.1269	1569	27/09/2015	-25.6	17.7	-27.1	19.3
MA_BIK_42	-5.2556	33.1320	1598	27/09/2015	-25.2	17.3	-26.8	18.9
MA_BIK_43	-5.2554	33.1323	1592	27/09/2015	-24.2	16.3	-25.8	17.9
MA_BIK_44	-5.2554	33.1325	1611	27/09/2015	-24.6	16.6	-26.1	18.3
MA_BIK_45	-5.2559	33.1332	1619	27/09/2015	-23.5	15.5	-25.1	17.1
MA_BIK_46	-5.2562	33.1341	1623	27/09/2015	-25.0	17.1	-26.6	18.7
MA_BIK_47	-5.2563	33.1351	1645	27/09/2015	-23.7	15.7	-25.2	17.3
MA_BIK_48	-5.2554	33.1336	1624	27/09/2015	-23.9	15.9	-25.5	17.6
MA_BIK_49	-5.2553	33.1334	1620	27/09/2015	-24.3	16.4	-25.9	18.0
MA_KOB_50	-5.2047	33.1072	2094	27/09/2015	-25.1	17.2	-26.7	18.8
MA_KOB_51	-5.2048	33.1068	2073	27/09/2015	-25.5	17.6	-27.0	19.1
MA_KOB_52	-5.2055	33.1060	2089	27/09/2015	-24.2	16.2	-25.7	17.8
MA_KOB_53	-5.2057	33.1055	2078	27/09/2015	-24.5	16.5	-26.0	18.1
MA_KOB_54	-5.2055	33.1054	2072	27/09/2015	-25.0	17.1	-26.5	18.7
MA_KOB_55	-5.2053	33.1051	2069	27/09/2015	-23.3	15.3	-24.9	16.9
MA_KOB_56	-5.2053	33.1046	2061	27/09/2015	-24.8	16.8	-26.3	18.4
MA_KOB_57	-5.2051	33.1043	2064	27/09/2015	-25.0	17.1	-26.5	18.7
MA_KOB_58	-5.2049	33.1043	2071	27/09/2015	-24.3	16.3	-25.8	17.9
MA_KOB_59	-5.2049	33.1040	2075	27/09/2015	-25.2	17.3	-26.7	18.9
MA_KOB_60	-5.2053	33.1040	2070	27/09/2015	-24.3	16.3	-25.8	17.9
MA_KAH_61	-5.2328	33.2472	1943	28/09/2015	-25.5	17.6	-27.0	19.1
MA_KAH_62	-5.2334	33.2488	1943	28/09/2015	-24.9	17.0	-26.5	18.6
MA_KAH_63	-5.2338	33.2492	1931	28/09/2015	-24.0	16.0	-25.6	17.7
MA_KAH_64	-5.2340	33.2499	1927	28/09/2015	-24.5	16.6	-26.1	18.2
MA_KAH_65	-5.2349	33.2505	1925	28/09/2015	-24.6	16.7	-26.2	18.3
MA_KAH_66	-5.2322	33.2486	1964	28/09/2015	-24.8	16.9	-26.4	18.5
MA_AZR_67	-5.2400	33.3283	1815	28/09/2015	-25.4	17.5	-26.9	19.1
MA_AZR_68	-5.2400	33.3283	1814	28/09/2015	-24.4	16.5	-26.0	18.1
MA_AZR_69	-5.2412	33.3270	1803	28/09/2015	-23.8	15.8	-25.3	17.4
MA_AZR_70	-5.2412	33.3262	1817	28/09/2015	-24.4	16.4	-25.9	18.0
MA_AZR_71	-5.2415	33.3257	1812	28/09/2015	-24.0	16.0	-25.5	17.7
MA_AZR_72	-5.2431	33.3250	1824	28/09/2015	-23.7	15.7	-25.3	17.4
MA_RIF_75	-5.0910	35.2493	1729	29/09/2015	-27.4	19.6	-28.8	21.1
MA_RIF_76	-5.2169	35.1843	1739	30/09/2015	-25.7	17.8	-27.2	19.4
UK_WB_01	-2.2331	51.6029	124	09/10/2014	-28.6	20.9	-30.1	22.4

Table 3 (continued)

Sample	Longitude	Latitude	Altitude	Collection date	Pollen $\delta^{13}\text{C}$ (‰)	Pollen $\Delta^{13}\text{C}$ (‰) <sup>a</sup>	Modelled sporopollenin $\delta^{13}\text{C}$ (‰) <sup>b</sup>	Modelled sporopollenin $\Delta^{13}\text{C}$ (‰) <sup>a</sup>
UK_WB_02	-2.2172	51.6052	124	09/10/2014	-28.3	20.5	-29.7	22.0
UK_WB_03	-2.2117	51.6081	124	13/10/2014	-27.7	19.9	-29.1	21.4
UK_WB_04	-2.2108	51.6082	137	13/10/2014	-27.7	19.9	-29.1	21.4
UK_WB_06	-2.2075	51.6116	140	13/10/2014	-29.3	21.6	-30.7	23.1
UK_MAN_07	-2.1894	53.4175	68	30/09/2015	-30.0	22.3	-31.4	23.8
UK_MAN_08	-2.1776	53.4093	34	03/10/2015	-28.9	21.2	-30.3	22.7
UK_MAN_09	-2.2315	53.4103	40	08/10/2015	-27.5	19.7	-29.0	21.2
US_BOS_01	-71.1215	42.2993	43	14/09/2015	-27.3	19.5	-28.8	21.0
US_BOS_02	-71.1220	42.2996	47	14/09/2015	-27.9	20.2	-29.4	21.7
FR_PAR_01	2.3598	48.8439	35	01/10/2015	-27.2	19.4	-28.6	20.9
FR_PAR_02	2.3598	48.8439	35	01/10/2015	-26.9	19.0	-28.3	20.6
FR_PAR_03	2.3598	48.8439	35	01/10/2015	-26.5	18.6	-28.0	20.2
FR_PAR_04	2.3598	48.8439	35	01/10/2015	-26.5	18.6	-27.9	20.2
FR_BOR_05	-0.6030	44.8547	23	01/10/2015	-26.3	18.4	-27.7	20.0
FR_BOR_06	-0.6030	44.8547	23	01/10/2015	-25.6	17.7	-27.1	19.3
FR_BOR_07	-0.6030	44.8547	23	01/10/2015	-25.2	17.3	-26.7	18.9
ES_PYR_01	-0.5526	42.5662	820	10/10/2015	-26.0	18.1	-27.4	19.6
ES_PYR_02	-0.5526	42.5662	820	10/10/2015	-25.1	17.2	-26.6	18.8

<sup>a</sup> For calculation of  $\Delta^{13}\text{C}$  see Materials and methods section.

<sup>b</sup> Modelled for all samples using the regression model (Fig. 3A): Sporopollenin  $\delta^{13}\text{C}$  = 0.9713 \* (Pollen  $\delta^{13}\text{C}$ ) - 2.2445.

with summer scPDSI ( $r^2 = 0.86, p < 0.0001$ ) (Fig. 6D). There is a similar strong significant relationship between sporopollenin  $\Delta^{13}\text{C}$  and aridity when calculated as  $AI_u = P/PET$  ( $r^2 = 0.80, p < 0.0001$ ); sporopollenin  $\Delta^{13}\text{C}$  decreases where there are higher levels of aridity.

At the regional scale, comparing only the sample locations in the Middle Atlas, they show a significant relationship with interpolated mean

annual precipitation and sporopollenin  $\Delta^{13}\text{C}$  ( $r^2 = 0.50, p < 0.03$ ) which is similar to the relationship observed across all samples using gridded CRU data. Aridity calculated for Middle Atlas locations using interpolated precipitation, with PET data from CRU as  $AI_u = P/PET$  showed a significant relationship with sporopollenin  $\Delta^{13}\text{C}$  ( $r^2 = 0.51, p < 0.03$ ) but was weaker than the relationship observed across all sample locations.

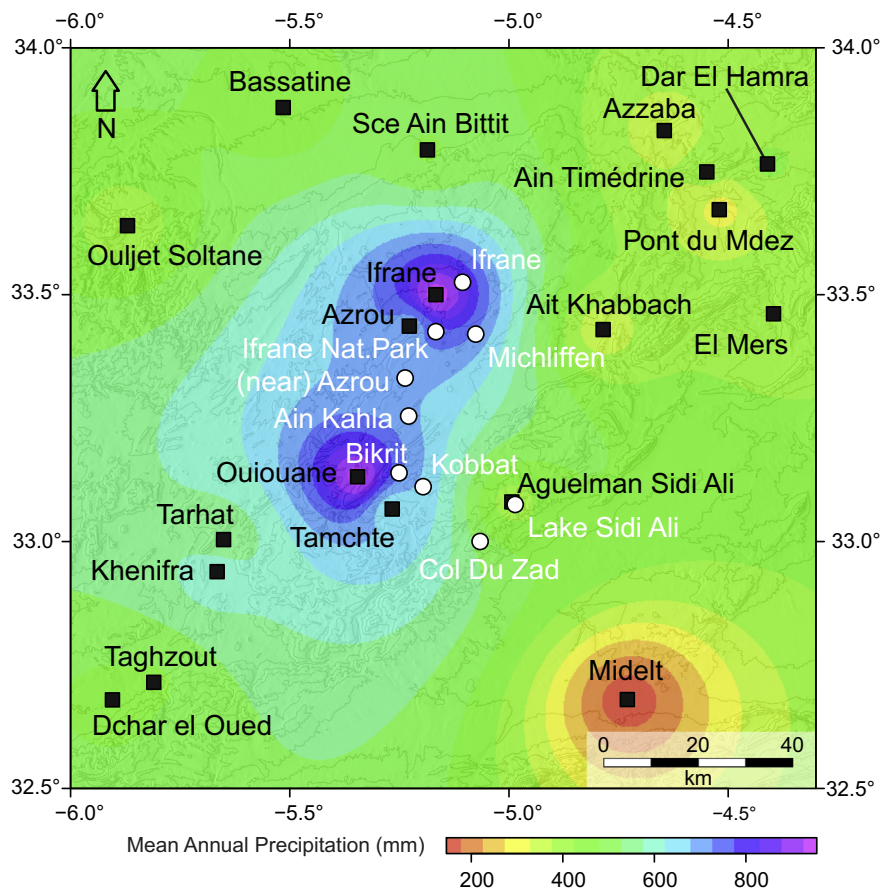
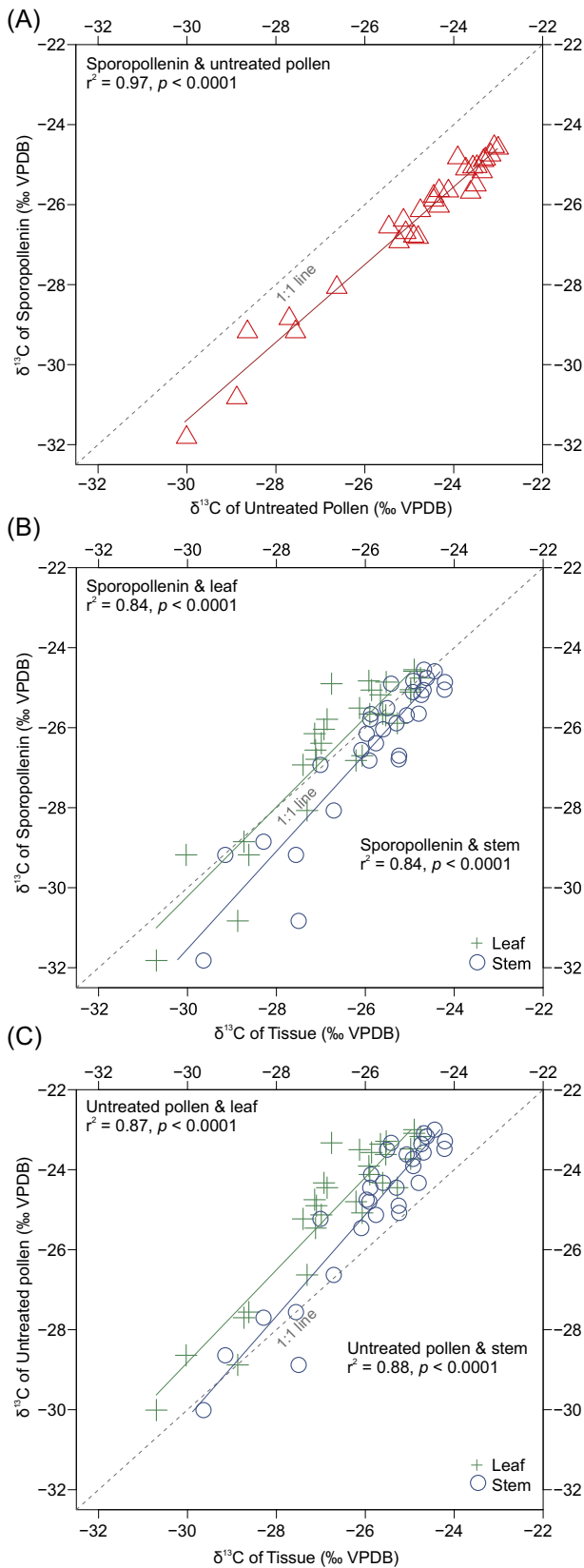


Fig. 2. Inverse Distance Weighted interpolation of mean annual precipitation for the wider Middle Atlas region. Sample locations (white circles) and climate stations (black squares) indicated.



**Fig. 3.** Biplots of  $\delta^{13}\text{C}$  results for untreated pollen, sporopollenin, leaf and stem tissue showing (A) sporopollenin versus untreated pollen  $\delta^{13}\text{C}$ , (B) sporopollenin versus leaf and stem tissue  $\delta^{13}\text{C}$ , and (C) untreated pollen versus leaf and stem tissue  $\delta^{13}\text{C}$  ( $n = 30$ ).

## 4. Discussion

### 4.1. Pollen and tissue isotopic composition

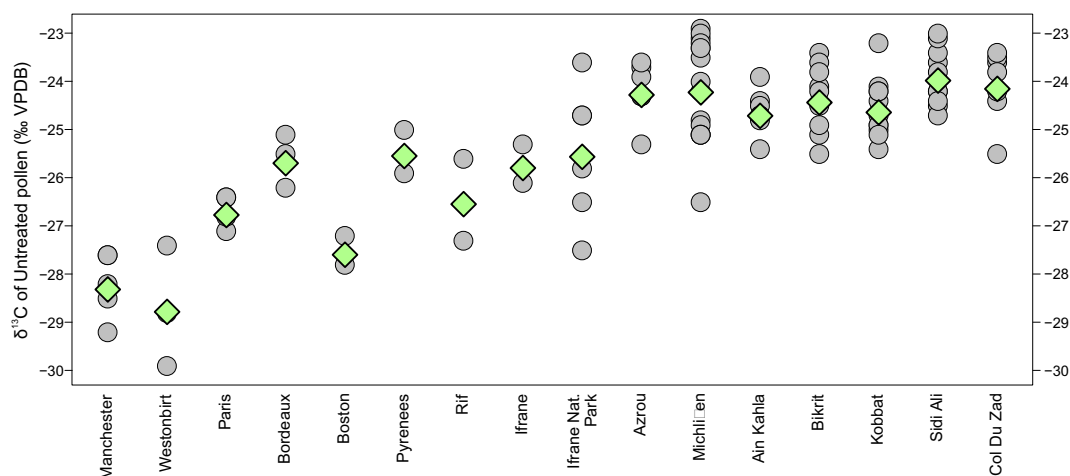
Carbon isotopic compositions of *Cedrus atlantica* pollen, sporopollenin and tissue are significantly related, suggesting pollen and sporopollenin  $\delta^{13}\text{C}$  could be used in place of leaf  $\delta^{13}\text{C}$  for understanding *C. atlantica* response to environmental conditions (Amundson et al., 1997; Jahren, 2004). The direction and extent of  $\delta^{13}\text{C}$  offsets between pollen, leaf and stem tissues for *C. atlantica* are consistent with other published studies for other species (Park and Epstein, 1960; Lowdon, 1969; O'Leary et al., 1981; Badeck et al., 2005; Cernusak et al., 2009). Influences on the  $\delta^{13}\text{C}$  between different tissues are debated, but may result from post-photosynthetic fractionation effects. Heterotrophic tissues (e.g. pollen and stem) may be supplied with photosynthate from different pools (Badeck et al., 2005; Cernusak et al., 2009; Brüggemann et al., 2011), and there may also be seasonal influence related to different development periods for different tissues (Francey et al., 1985).

We found that *Cedrus atlantica* sporopollenin  $\delta^{13}\text{C}$  has a strong linear relationship with untreated pollen  $\delta^{13}\text{C}$  ( $r^2 = 0.97$ ) depleted in  $^{13}\text{C}$  by an average 1.5‰. Findings are similar to those observed for other woody species; *Betula pendula*, *Pinus sylvestris*, and *Picea abies* ( $r^2 = 0.93$ ) (Loader and Hemming, 2000), *Nothofagus* ( $r^2 = 0.64$ ) (Griener et al., 2013), and some herbaceous species; *Ambrosia* species ( $r^2 = 0.85\text{--}0.92$ ) and *Artemisia* species ( $r^2 = 0.85\text{--}0.99$ ) (Nelson, 2012). Sporopollenin forms the exine and is the main component of pollen comprising 65–80% of the grain (Brooks and Shaw, 1978; Loader and Hemming, 2000; Wiermann et al., 2001). As it is the only part which survives in the fossil record, it is important for palaeoclimatic reconstruction that the isotopic composition of *C. atlantica* sporopollenin can be accurately determined from untreated pollen. The remaining parts of the grain comprise several constituents which may include cellulose, polysaccharides and xylose sugars (Brooks and Shaw, 1978) and are enriched in  $^{13}\text{C}$ . Sporopollenin is thought to consist largely of aliphatic long-chains and phenolic aromatic moieties which largely comprise p-coumaric acid and ferulic acid (Bergen et al., 2004; Blokker et al., 2006), although its exact chemical composition is unknown. Nuclear magnetic resonance (NMR) on sporopollenin (Guilford et al., 1988; Hemsley et al., 1992, 1993) would suggest that it is not a unique homogeneous substance, but instead comprises a mixture of biopolymers deriving from fatty acids. It is not known which constituents of sporopollenin are similar between species, and composition of pollen grains between species may vary. Studies using Fourier transform infrared spectroscopy (FTIR) suggest chemical differences between taxa may result from differences in aliphatic chain lengths (Julier et al., 2016). The results we report are consistent with the differing sporopollenin and untreated pollen  $\delta^{13}\text{C}$  offsets reported in the literature, and support a significant difference between sporopollenin and other components of pollen.

Overall, if the isotopic offsets are consistent then using the  $\delta^{13}\text{C}$  values of one tissue as a proxy for another is possible. For example, Jahren (2004) suggested that the  $\delta^{13}\text{C}$  value of bulk pollen samples could infer the  $\delta^{13}\text{C}$  value of past plant communities to within 1.5‰, and our results are consistent with this finding. In addition, as our sporopollenin  $\delta^{13}\text{C}$  values closely match leaf  $\delta^{13}\text{C}$  values for *Cedrus atlantica*, this suggests that pollen from the fossil record would be a strong predictor for leaf  $\delta^{13}\text{C}$  of past *C. atlantica* populations where values could be used interchangeably.

### 4.2. Isotopic variability within sample locations

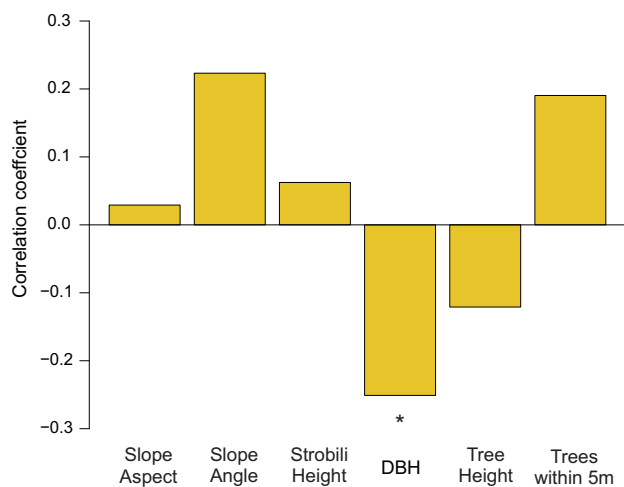
Local environmental factors within sample locations and physiological differences between specimens may account for some of the variation in isotopic composition between individual samples, for example micro-climate variations, localised water availability variation, and



**Fig. 4.** Dot plot of untreated pollen  $\delta^{13}\text{C}$  values grouped by sample location (grey circles) with mean  $\delta^{13}\text{C}$  for each location indicated by green diamonds, ordered along a north to south gradient ( $n = 93$ ).

nutrient availability, (e.g. Zhang and Marshall, 1994; Griffiths et al., 1999; Warren et al., 2001). At some of our sample locations we observe quite large intra-site variability, up to 3.8‰, although this is consistent with other findings (Körner et al., 1991; Van de Water et al., 2002; King et al., 2012). We found a positive correlation with slope angle and  $\delta^{13}\text{C}$ , and the number of trees within 5 m of the sampled specimen and  $\delta^{13}\text{C}$  (Fig. 5). We suggest these micro-scale environmental factors could influence the moisture availability to individual trees which are growing under the same climate conditions, leading to less discrimination against  $^{13}\text{C}$  under drier conditions as steeper slopes could experience increased water runoff and higher heating by insolation, while a higher density of surrounding trees would increase competition for available water.

Tree height and DBH have a negative correlation with pollen  $\delta^{13}\text{C}$ . These growth factors are non-linearly correlated with tree age, which suggests that older trees may exhibit more negative  $\delta^{13}\text{C}$  compared to younger trees under the same conditions, and may therefore be better adapted to moisture stress. Linares et al. (2013) found a similar result with respect to growth of *Cedrus atlantica* suggesting that climate-growth relationships are dependent on age. Younger *C. atlantica* trees showed a slight increase in precipitation sensitivity compared with older trees, which Linares suggests is due to higher xylem hydraulic safety (Ryan et al., 1997) in older trees, but their larger size increases



**Fig. 5.** Correlations between  $\delta^{13}\text{C}$  and micro-scale sample site or specimen characteristics for the samples collected in the Middle Atlas, Morocco. \* indicates statistically significant correlation ( $p < 0.05$ ) ( $n = 72$ ).

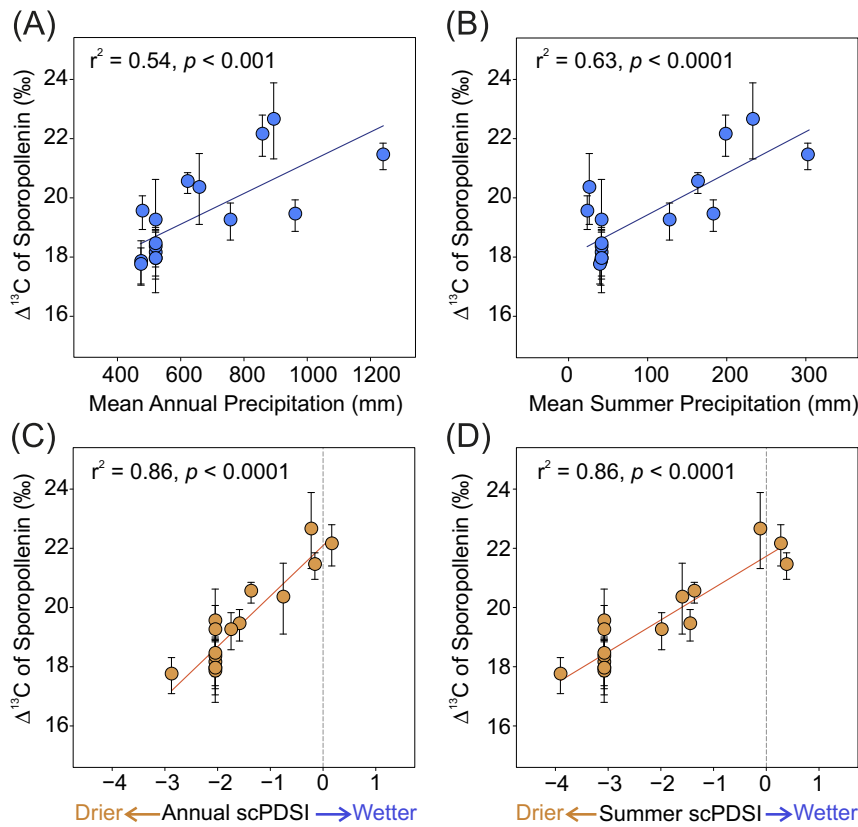
respiration costs (Hunt et al., 1999) making them more sensitive to temperature. In a meta study, Fardusi et al. (2016) found  $\delta^{13}\text{C}$  was positively associated with increased biomass production in woody plants, however this trend was not significant for the Mediterranean, and the association was negative for some species such as *Pinus halepensis* (Ferrio and Voltas, 2005). While those studies involved measurements on woody tissue, our results suggest that climate age-related growth-limiting factors may also be reflected in the  $\delta^{13}\text{C}$  signal of modern pollen.

#### 4.3. Isotope discrimination and moisture availability

*Cedrus atlantica* sporopollenin  $\Delta^{13}\text{C}$  demonstrates a significant relationship with mean annual precipitation and, especially during the summer development season for pollen. It is comparable to relationships between *Nothofagus* sporopollenin  $\Delta^{13}\text{C}$  and precipitation for its development season ( $r^2 = 0.72$ ) (Griener et al., 2013), and for  $\Delta^{13}\text{C}$  from tree-rings of *Pinus halepensis* and mean annual precipitation ( $r^2 = 0.60$ ) which grows in similar semi-arid conditions (Del Castillo et al., 2015). Furthermore, the sensitivity of discrimination in *P. halepensis* wood tissue (0.47‰ per 100 mm precipitation) is remarkably similar to *C. atlantica* sporopollenin (0.52‰ per 100 mm precipitation), and much greater than that observed in *Quercus ilex* (~0.17‰ per 100 mm precipitation) (Ferrio et al., 2003). This comparison supports the important stable carbon isotope signature of moisture availability in shallow-rooting Mediterranean conifers, and reveals the high sensitivity of *C. atlantica* sporopollenin  $\Delta^{13}\text{C}$  to drought stress.

However, we found there is a stronger relationship between sporopollenin  $\Delta^{13}\text{C}$  and aridity, as measured using two indexes: scPDSI and  $\text{AI}_w$ . This may demonstrate that moisture stress and soil-moisture availability particularly in semi-arid areas could be a strong driver of *Cedrus atlantica* response to climate. For semi-arid areas, temperature can act as a key control over forest drought stress (Williams et al., 2012), and may be the primary limiting growth-factor (Fensholt et al., 2012), as well as driving increased evaporative loss in open environments (Snyder and Tartowski, 2006). Changes to relative humidity and increasing water pressure deficit (VPD) would decrease  $\Delta$ , as stomatal conductance slows to reduce water loss by restricting leaf transpiration (Zhang and Nobel, 1996; Bowling et al., 2002). The shallow root systems of *C. atlantica* (Aussenac, 1984) which limit access to deep-water, may cause a reduction in stomatal conductance when soil moisture availability is low. This reduces  $\text{CO}_2$  internal partial pressure ( $C_i$ ) causing a steady enrichment of  $^{13}\text{C}$  within the leaf (i.e. reduced  $\Delta$ ) which leads to enrichment of  $^{13}\text{C}$  in other plant biomass such as pollen (Farquhar and Sharkey, 1982; Marshall et al., 2007; Lambers et al.,



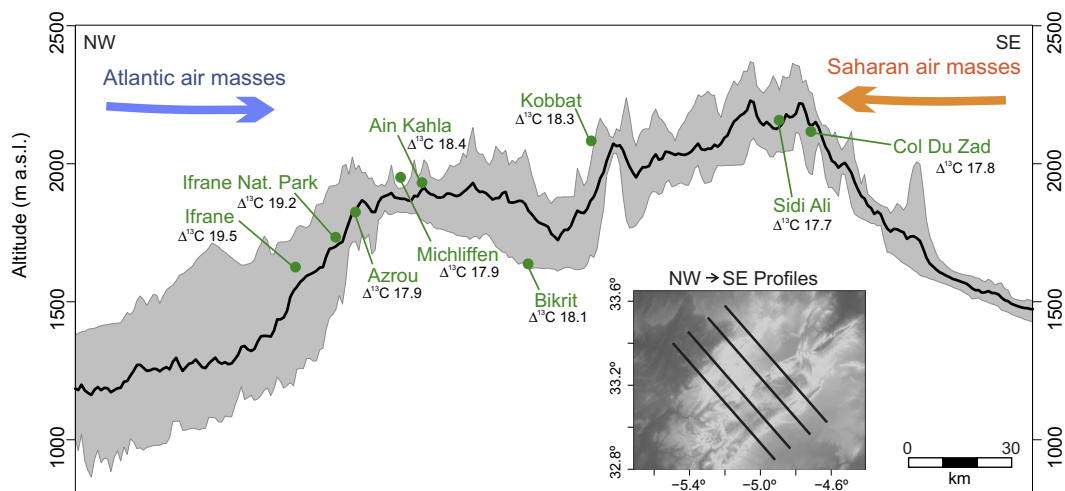


**Fig. 6.** Biplots showing mean modelled sporopollenin  $\Delta^{13}\text{C}$  by sample location versus precipitation (CRU 30-year mean) and self-calibrating Palmer Drought Severity Index (30-year mean) (Dai, 2011). (A) Mean annual precipitation, (B) mean summer precipitation, (C) mean annual scPDSI, and (D) mean summer scPDSI. Error bars indicate standard deviation. Regression models: (A)  $\Delta^{13}\text{C} = 0.0052 * P_{\text{ann}} + 15.996$ , (B)  $\Delta^{13}\text{C} = 0.014 * P_{\text{summ}} + 18.025$ , (C)  $\Delta^{13}\text{C} = 1.7042 * \text{scPDSI}_{\text{ann}} + 22.09$ , (D)  $\Delta^{13}\text{C} = 1.0756 * \text{scPDSI}_{\text{summ}} + 21.732$ .

2008). *Cedrus atlantica* already demonstrates growth-response sensitivity to temperature through its effects on transpiration in older specimens (Linares et al., 2013). The stronger relationships we observe with aridity, recorded in the sporopollenin  $\Delta^{13}\text{C}$  signal, may result from these indexes accounting for evaporation and reduced soil-moisture, which appear to be a key control on *C. atlantica* response. An important role of aridity has similarly been highlighted for *Pinus halepensis* and *Quercus ilex* in water-stressed settings (Ferro et al., 2003).

The more modest relationship we observe with precipitation may also be due to the poor availability of precipitation data and low density

of rainfall stations in the Middle Atlas, as they do not fully capture complex microclimatic patterns in the region. Our interpolation of precipitation (Fig. 2) highlights that precipitation varies significantly across the Middle Atlas. Weather stations at Ifrane in the northwest Middle Atlas record double the amount of annual precipitation compared to the station at Lake Sidi Ali in the south. Climate regimes in the region are strongly influenced by Atlantic and Saharan air masses, and the NW to SE precipitation gradient reflects the prevailing westerly Atlantic moisture-bearing air masses and orographic effects on the windward margin of the Atlas chain (Fig. 7). There are also seasonal differences across the region; for example, Ifrane has very high winter precipitation



**Fig. 7.** Location of Middle Atlas sample locations along a NW to SE elevation profile showing mean sporopollenin  $\Delta^{13}\text{C}$  of each location. Average elevation (thick black line) and min/max elevation (grey shaded area) shown for the 4 swath profiles (inset box).

and low summer precipitation, while Sidi Ali has low winter precipitation, but slightly higher summer precipitation than Ifrane, associated with convective storms along the Atlas–Saharan margin (Born et al., 2010). The prevailing precipitation influences on moisture availability appear to be reflected in the  $\Delta^{13}\text{C}$  values of sporopollenin across the sample locations (Fig. 7). Moving from Northwest to Southeast,  $\Delta^{13}\text{C}$  values generally become lower ( $r_s = 0.74$ ,  $p < 0.02$ ), corresponding to a decrease in available moisture from precipitation and increases to evaporative loss from higher temperatures.

#### 4.4. Challenges and applications for pollen geochemistry

There is growing interest in analysis of pollen geochemistry, e.g. for quantitative climate and environmental information, and species-level identification (Pappas et al., 2003; Julier et al., 2016; Zimmerman et al., 2016). However, isolation of sufficient pollen grains from sediment archives for geochemical analysis can be labour and time intensive (Jahren, 2004), and traditional chemical methods to concentrate pollen could result in isotopic contamination (Amundson et al., 1997; Loader and Hemming, 2000). Methods employing micro-sieving and density separation for concentration of pollen for radiocarbon dating can produce pollen-rich concentrates (Fletcher et al., 2017), following which, specific pollen species can be more easily isolated using manual micro-manipulation. Furthermore, flow cytometry offers the potential for automated sorting and separation of pollen by species (Mitsumoto et al., 2010; Tennant et al., 2013; Yelina et al., 2013) based on the auto-fluorescence of the grains (Yeloff and Hunt, 2005). Isotopic analysis using a spooling-wire interface (Nelson et al., 2007) also reduces the number of pollen grains required for analysis, suggesting these methods and analytic techniques can make geochemical pollen studies more practical, reducing the time required for sample preparation.

The isotopic composition of pollen could also be used to provide precipitation information in regions where precipitation data availability is poor or unavailable. For example, carbon isotopic values from tree-ring networks have been used to reconstruct and map precipitation (Leavitt et al., 2007; Del Castillo et al., 2013). Since our results show strong relationships between the isotopic composition of pollen and other plant tissue for *Cedrus atlantica*, this technique could be adapted to use pollen as a tool for mapping precipitation and drought stress in place of tree-rings. For Morocco, this would allow mapping of summer conditions across the Middle Atlas where climate monitoring stations are sparse.

Overall, the evidence presented indicates that isotopic composition of *Cedrus atlantica* sporopollenin is a strong indicator of moisture availability and moisture stress, suggesting isotopic analysis of fossil pollen would be a feasible technique for reconstruction of past climatic conditions in the Middle Atlas. As a result of climate change, warmer temperatures and increasing drought are predicted for the region (Born et al., 2008; Solomon et al., 2009). Studies of past vegetation–climate dynamics are therefore important for an improved understanding of past climate change and predicting future climate change. There is good potential for developing Holocene applications of this technique as *C. atlantica* is well represented in many archives (Lamb et al., 1991; Lamb et al., 1995; Lamb and van der Kaars, 1995; Cheddadi et al., 2009; Zielhofer et al., 2017), and carbon isotopic signals of drought stress could also be used to test and refine quantitative climate reconstructions based on transfer-function approaches to pollen assemblage composition (Cheddadi et al., 1998; Nourelbait et al., 2016). Further, the observed isotopic relationships between sporopollenin and stem tissue suggest that isotope data from fossil pollen studies could also be integrated with *C. atlantica* tree-ring series for the last 900 years.

## 5. Conclusions

The stable carbon isotopic compositions of *Cedrus atlantica* pollen, sporopollenin, leaf and stem tissue are strongly related. This relationship is strongest for untreated pollen and sporopollenin, which have a

significant positive relationship suggesting sporopollenin  $\delta^{13}\text{C}$  values can be accurately estimated from untreated pollen. We found significant relationships between carbon isotopes in *C. atlantica* and measures of moisture availability and moisture stress. Sporopollenin  $\Delta^{13}\text{C}$  (derived from pollen  $\delta^{13}\text{C}$  values) is related to mean annual and summer precipitation, showing increased discrimination with greater precipitation levels. A stronger relationship exists between sporopollenin  $\Delta^{13}\text{C}$  and scPDSI aridity;  $\Delta$  is higher where aridity decreases. This finding suggests *C. atlantica* is responding to summer moisture stress as the aridity indexes better reflect the overall availability of moisture due to the combined role of precipitation supply and loss through enhanced evaporation, especially important in semi-arid areas. Our results also suggest that age-related drought stress resilience may be recorded in the carbon isotope composition, as older trees appear to discriminate against  $^{13}\text{C}$  more than younger trees. Overall, our study shows that *C. atlantica* sporopollenin  $\Delta^{13}\text{C}$  is a strong indicator of moisture availability and drought stress, due to a combination of macro- and micro-climatic and environmental influences (e.g. slope angle and competition for water from surrounding trees), and could be used for palaeoclimatic reconstruction in Northwest Africa.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.revpalbo.2017.04.008>.

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