RESEARCH ARTICLE

Pollen-chemistry variations along elevation gradients and their implications for a proxy for UV-B radiation in the plant-fossil record

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Abstract

1. Research indicates that phenolic compounds (e.g. *para*-coumaric acid) found within pollen grains may be useful as a proxy to reconstruct the UV-B radiation received at the Earth's surface in the geological past. However, application of this method to the plant-fossil record currently relies on a series of untested assumptions surrounding the ecological factors driving the response of pollen grains in the contemporary environment.

2. Here, we investigate the relationship of *Pinus* spp. pollen to UV-B radiation using individuals of five populations sampled from three elevation gradients across Europe. We develop a novel radiation-modelling approach, which allows us to estimate the UV-B radiation dose of individual trees, weighted by different UV-B action spectra. We then use linear mixed-effects modelling to investigate: (a) whether the variations in UV-B-absorbing compounds in *Pinus* pollen are best described by models using coarser (subgenus) or finer (population) taxonomic levels; and (b) the duration of the period of accumulation of UV-B-absorbing compounds in pollen, ranging from 8 to 28 days.

3. Our results demonstrate an overall positive relationship between *para*-coumaric acid and UV-B radiation, best described by applying a UV-B-accumulation period spanning 12–19 days. However, we also show clear evidence for population-level factors influencing this relationship across the study locations.

4. *Synthesis*. Our multidisciplinary approach, which combines expertise from palaeoecology, plant physiology and atmospheric physics, provides clear evidence that pollen-grain chemistry is subject to population-level variations. We suggest that quantitative reconstructions of long-term changes in springtime UV-B radiation are still achievable using fossil reconstructions, but only with careful consideration of the factors leading to pollen representation in sediments. Future improvements are dependent on mechanistic understanding of the local factors which

mediate the UV-B response across different populations, and on upscaling knowledge at the plant level to incorporate longer-term chemical variations represented within sediment samples.

KEYWORDS

palaeoecology and land-use history, *para*-coumaric acid, *Pinus* spp. pollen, plant–climate interactions, UV-B radiation, UV-B-absorbing compounds

1 | INTRODUCTION

Excess ultraviolet-B radiation (UV-B, ultraviolet, 280–315 nm) is known to induce a stress response in plants and has effects on the biosphere at the level of genes, species and ecosystems (Neale et al., 2021). Although the Montreal Protocol has been successful in both reducing the abundance of ozone-depleting substances in the atmosphere and allowing the subsequent recovery of stratospheric ozone (Solomon et al., 2016), there is widespread interest in understanding how variations in atmospheric-ozone concentrations, and the associated changes in UV-B radiation, have affected plant and ecosystem functioning in the geological past (Jardine et al., 2016, 2020; Lomax et al., 2008; Rozema et al., 2001, 2009; Willis et al., 2009). Yet despite the fact that UV-B is an essential variable for understanding dynamics of the Earth system, quantitative reconstructions of UV-B radiation beyond the instrumental record have remained elusive. This means that we lack robust empirical observations of UV-B radiation across key periods in the Earth's geological history where variations in ozone may have played a role in biosphere dynamics and in climatic and ecological change.

An emerging technique has proposed to use the chemical response of pollen grains as a tool to reconstruct the UV-B radiation received by plants (Jardine et al., 2016, 2020; Lomax et al., 2008; Rozema et al., 2009; Willis et al., 2011). Pollen-grain exines are made of sporopollenin, a complex biomolecule, which is partially composed of phenolic building blocks within a long-chain fatty-acid matrix (Leeuw et al., 2006; Li et al., 2019). Phenolic compounds, such as *para*-coumaric and ferulic acids, are effective absorbers of UV-B radiation and it is thought that they act to protect the genetic material within the pollen grain from UV-B radiation in the natural environment. Sporopollenin is also a chemically stable compound which is preserved in fossil sequences and can remain resistant to corrosion over millions of years (Fraser et al., 2012). Thus, a number of studies have investigated whether the chemical composition of pollen may be used as a proxy to reconstruct UV-B radiation from sediments on decadal to multi-millenial timescales (Fraser et al., 2011; Jardine et al., 2016; Lomax et al., 2008; Rozema et al., 2001; Willis et al., 2011).

However, although phenolic compounds are generally found in higher abundances in the pollen of plants grown under high UV-B or solar radiation (Fraser et al., 2011; Jardine et al., 2016; Lomax et al., 2008; Rozema et al., 2001; Willis et al., 2011), current understanding is based on a mixture of greenhouse and field-based studies, which have followed a broad set of experimental designs. Different UV-B exposure times, and even different wavelengths of UV-B or solar radiation have been used to investigate the pollen-chemical response (Seddon et al., 2019), whilst other studies have combined responses from multiple species along large latitudinal (e.g. Jardine et al., 2016; Willis et al., 2011) or elevational gradients (e.g. Lomax et al., 2012). One consequence of this is that it is impossible to make systematic comparisons between studies, particularly for species groups whose taxa are prevalent in palaeoecological records in lake and bog sediments (e.g. such as wind-pollinated trees). The overall result is that a quantitative understanding of the dose-response relationship between UV-B radiation and the accumulation of phenolic compounds in pollen has not yet been achieved. The reconstructions that have been developed so far using the pollen-chemistry approach are dependent on a series of ecological assumptions that remain poorly understood.

For example, current calibration models rely on the assumption that the response of pollen to UV-B radiation is consistent across different species within a single genus (e.g. Jardine et al., 2016; Willis et al., 2011). This assumption is based on evidence which shows that the genetic mechanisms used in the perception of UV-B radiation in *Arabidopsis thaliana* are similar to those in algae and mosses (Lomax & Fraser, 2015; Rizzini et al., 2011). However, UV-B responses to a number of other plant processes (e.g. pollen-tube growth length) are highly variable across different plant groups (Torabinejad et al., 1998), and sensitivity of a number of key growth parameters (e.g. growth height, leaf production, leaf length) to UV-B radiation can differ depending on environmental context (Robson et al., 2014). Furthermore, in studies working in parallel to research related to proxy development for UV-B radiation, results have demonstrated that the chemical constituents of sporopollenin (in terms of both the relative quantities and types of phenolic and other compounds present) can vary between families (Zimmermann & Kohler, 2014), genera and species (Jardine et al., 2019; Julier et al., 2016; Muthreich et al., 2020). Since specific biochemical signatures are found within certain pollen types, it is unclear whether calibration models developed for one taxon can be directly applied to other taxa. Furthermore, if species-level variations are important when considering UV-B radiation responses, then this may bias reconstructions of UV-B radiation based on chemical variations in pollen because fossil reconstructions generally standardise pollen types to their corresponding genus or family. In this case, reconstructed variations in UV-B-absorbing compounds could reflect cryptic community

turnover, rather than UV-B effects, if dose-response relationships are not consistent at the taxonomic level that is possible to be observed in the fossil record.

Indeed, it may even be necessary to consider the factors which influence the biochemical response of pollen to UV-B radiation between different populations (Bell et al., 2018; Diehn et al., 2018). Recent evidence indicates that, despite growing in different radiation environments, the infrared absorbance spectra obtained from pollen grains from *Cedrus atlantica* sampled from botanic gardens closely resembled their origin populations (Bell et al., 2018). These effects potentially indicate the importance of local adaptation in the chemical composition, likely as a result of differential selection pressures, and highlight that phenotypic variation in the chemical response of pollen grains might also exist at the population level (Benito-Garzón et al., 2011). There is widespread evidence for population-level variation in numerous tree species (Rehfeldt et al., 2002; Savolainen et al., 2007), and ecologists have recently noted the challenges that population variation can create in terms of developing predictive models to environmental change. In response to these challenges, an emerging set of predictive models are currently under development that are able to incorporate population-level interactions (Benito Garzón et al., 2019). Similarly, palaeoecologists may need to consider how population-level variation might influence models which aim to reconstruct UV-B radiation in the past. The first step towards addressing this question would be to investigate the potential existence of population-level variation within a pollen type that is most relevant for palaeoecological research.

An additional uncertainty relates to the representative exposure period over which phenolic compounds accumulate within the pollen grain, and which best explains the response of UV-B-absorbing compounds to solar UV-B radiation (Seddon et al., 2019). Studies on the chemical responses of plants indicate that the abundance of phenolic compounds can vary on daily, seasonal and annual timescales (Barnes et al., 2017). Whether pollen-grain chemical variability reflects weekly or seasonal variations of UV-B radiation is an important question because it will determine whether the proxy can represent a signal of short-term (e.g. interannual changes related to cloud cover) compared to longer-term (e.g. climatological mean) variations. This has yet to be tested formally in the context of palaeocological UV-B research, particularly for pollen types that are representative of the fossil record.

Here we investigate how the accumulation of *para*-coumaric acid varies in pollen from individuals of *Pinus sylvestris*, *P. mugo*, *P. mugo*

spp. *uncinata* and *P. cembra* along elevation gradients in three different sites across Europe. We develop a novel radiation-modelling approach, which allows us to estimate the UV-B radiation dose of individual trees, weighted by different UV-B action spectra. We then use linear mixed-effects modelling to investigate two central assumptions underlying previous pollen-based reconstructions of UV-B radiation: (a) whether the variations in UV-B-absorbing compounds in *Pinus* pollen are best described by models using coarser (subgenus) or finer (population) taxonomic levels; and (b) the duration of the period of accumulation of UV-B-absorbing compounds in pollen, ranging from 8 to 28 days. We use this information to estimate an overall response rate to UV-B radiation for the accumulation of the *para*-coumaric acid in *Pinus* pollen and discuss the implications of our findings with respect to developing a proxy for UV-B radiation based on the chemical variations of pollen observed in the fossil record.

2 | MATERIALS AND METHODS

2.1 | Pollen collection

Pollen was sampled from individuals of *Pinus sylvestris*, *P. mugo*, *P. mugo* spp. *uncinata* and *P. cembra* from natural populations along elevation gradients in three locations up to the tree line (Table 1). Three of the taxa (*Pinus sylvestris*, *P. mugo*, *P. mugo* spp. *uncinata*) belong to the same sub-genus (*Pinus*), and their pollen grains are impossible to separate at the species level based on pollen-morphological features alone (Beug, 2004). Therefore, palaeoecologists often harmonise pollen grains into a single pollen type (e.g. *Pinus sylvestris* type, Giesecke et al., 2019). *P. cembra* pollen grains are distinct from the subgenus *Pinus* under a light microscope and so would likely be separated from the other *Pinus* taxa in a palaeoecological analysis.

We sampled *Pinus* pollen during the time of peak dehiscence from a total of 59 individuals and estimated UV-B exposure using a novel method which combines UV-B radiation sensors and spectral modelling (see below). Note that, originally, ten additional *P. sylvestris* trees and three individuals of *P. mugo* were also monitored from the Patcherkofel slope in Innsbruck, and single individuals of *P. sylvestris* and *P. mugo* spp. *uncinata* were monitored in the town of Setcases, close to the eastern Pyrenees. However, since these individuals were located in horticultural settings (e.g. botanic gardens or parks), their provenance is not known. Because

TABLE 1 Overview of the taxa sampled in this study

Species	Subgenus	No. individuals	Minimum elevation (m)	Maximum elevation (m)	Mean latitude (°)
Pinus cembra	Strobus	30	1,866	2,191	47.2
Pinus mugo	Pinus	33	1,616	1,808	47.3
Pinus sylvestris	Pinus	30	94	360	60.3
P. sylvestris	Pinus	77	490	1,122	42.3
P. mugo ssp. *uncinata*	Pinus	58	1,289	2,250	42.4

provenance may affect the overall response of pollen chemistry when considering responses across latitudinal gradients (Bell et al., 2018), these individuals were not considered in the main part of this study.

All trees were checked regularly (every 2 to 7 days) for pollen-cone formation starting from March 2018 (Innsbruck, Eastern Pyrenees) and April 2019 (Bergen). Pollen was sampled at the moment that the inflorescences from the sun-exposed side of the tree had opened to shed pollen. Four to six male pollen cones per tree were collected into paper bags, dried for 24 hr at room temperature and then stored at −22°C before analysis.

2.2 | Quantification of *para*-coumaric acid

Pyrolysis Gas Chromatography Mass Spectrometry (py-GC-MS) was used to quantify the abundance of *para*-coumaric acid (Blokker et al., 2005). Internal standards were prepared and used to calculate the quantity of *para*-coumaric acid per pollen grain (in units of ng/grain) following the approach of Seddon et al. (2017), and a separate calibration sample set was run to correct for differences between sample batches. See Supporting Information 1 for more details.

2.3 | Quantification of UV-B exposure

We estimated surface UV-B irradiance for individual trees via a combination of UV-B radiometers and UV-B modelling (Figure 1). A Skye Instruments UV-B (SKU 430) radiometer, with peak wavelength response at ~300 nm, was placed close to each population on across the sample grid (Supporting Information 2). The radiometers were calibrated using a Maya 2000 Pro (Ocean Optics) CCD array spectrometer which was recently calibrated for measurements of solar radiation over the spectrum 290–900 nm (Aphalo et al., 2016). The integration time of spectrometer readings was optimised to maximum sensitivity of the array in the UV-B region using bracketing, and paired dark and UV-filtered corrections, to account to stray light in post-processing (following Hartikainen et al., 2018).

These UV-B radiometers were used to develop a cloud-correction factor for each tree location. The cloud-correction factor was then applied to the modelled clear-sky surface UV-B irradiance spectra and doses, estimated using AccuRT, a multi-stream radiative-transfer code (Hamre et al., 2016; Stamnes & Stamnes, 2016). The AccuRT model included atmospheric ozone-column estimates, loess smoothed to hourly intervals, from the satellite-borne total-ozone mapping spectrometer (TOMS; Figure 1).
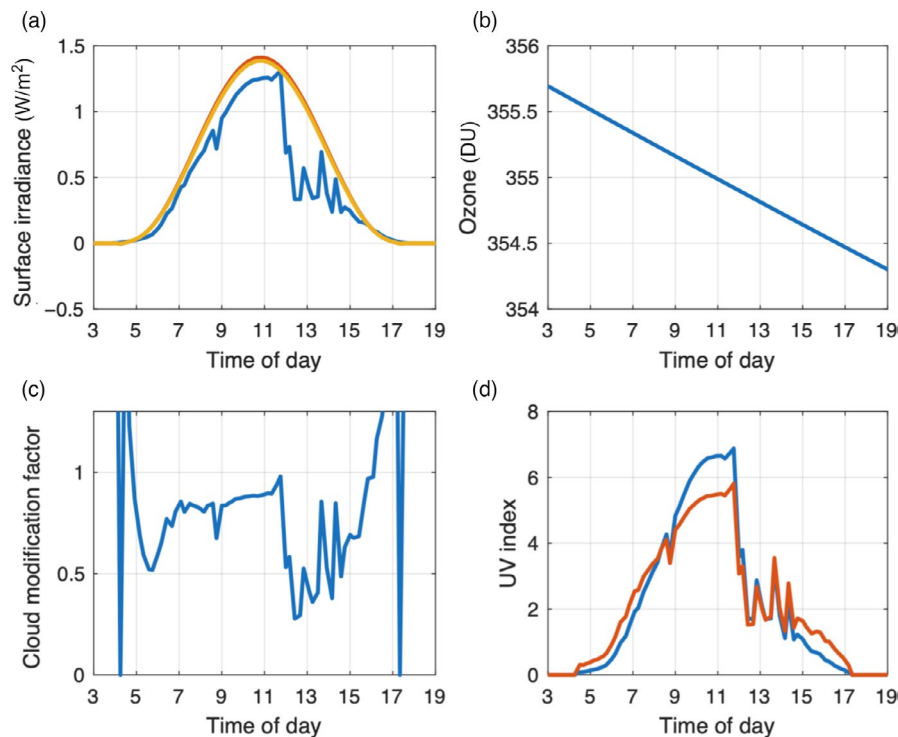
FIGURE 1 Example of measured and modelled daily-varying irradiances with corresponding parameters. (a) Instrument-specific surface irradiance with a response function ranging from 280 to 315 nm at full-width half maximum: (blue) measured data under broken clouds at the site of radiometer, (red) modelled for clear sky at instrument site, (yellow) modelled data for clear sky at individual tree location (b) Variation in daily ozone-column thickness measured by the satellite-borne total ozone mapping spectrometer (TOMS). (c) Cloud-modification factor obtained from (a), used to scale modelled clear-sky data at the individual tree location (d) Ultraviolet radiation doses: (blue) UV index calculated for the CIE-standard McKinlay-Diffey erythemal action spectrum (shown for comparison, not used in this study), (red) same calculations as for the UV index, but with the Flint-Caldwell plant-growth action spectrum. Example of received UV radiation for the tree PS4 (*Pinus sylvestris*) from the Eastern Pyrenees on 15 April 2018

For each day, two different UV-B radiation doses were calculated: (a) the radiant exposure, which is obtained by integrating the weighted-mean irradiance (280–315 nm) over the period when the sun is above the horizon, and (b) the same daily-integrated irradiance, but now with each irradiance spectrum weighted by the plant-growth action spectrum (Flint & Caldwell, 2003a, 2003b). Different accumulation periods were calculated from 28 to 8 days. In 2019, the UV-B radiometer from the Bergen site was installed on 3 May 2019, but a number of trees produced pollen earlier than expected so that the full 28–day period could not be calculated. Therefore, we developed a calibration model using photosynthetically active radiation (PAR) measured at 5-min intervals from a nearby meteorological station and then applied this calibration model to estimate the daily maximum UV-B for the relevant accumulation period in 2019 (Supporting Information 3). In addition, finding a suitable location for the radiometer close to the *Pinus mugo* sampling site in Innsbruck was challenging, and we were forced to compromise on a location which resulted in a detectable shading effect on the radiometer in the data between 08:55 and 11:20 a.m. Therefore we made a linear interpolation between the two time measurements from before and after the shading period. These data were then used to estimate the cloud-correction factor for this site (Supporting Information 3).

2.4 | Statistical modelling

We used linear-mixed effects models to test the relationship between *para*-coumaric acid and UV-B radiation over different exposure periods and weighted (using the plant-growth action spectrum) and unweighted UV-B doses. We log-transformed the response variable (quantity of *para*-coumaric acid per pollen grain) and used a Gaussian error distribution. Note that we initially used a Gamma distribution with a log-link function, but these had convergence issues for poorly fitting models. A comparison of the results of the optimal models using both model types showed similar results.

We fit models with varying exposure periods ranging from 8 to 28 days prior to pollen production. For each UV-B exposure period and for each UV-B dose we fit four different models: a null model (an intercept-only model), a fixed-effects only model, a random-effects model with a random-intercept, and a random-effects model with both a random slope and intercept, estimating parameters using the restricted maximum likelihood. In all models the fixed effect was the UV-B radiation dose, whilst the random effects were defined at either the subgenus (i.e. *Pinus* or *Strobus*) or population level (e.g. *Pinus sylvestris*: Bergen, *Pinus mugo*: Innsbruck, etc.). If evidence favoured a model with random effects at the subgenus level, then this would indicate that integration of pollen types across species and populations is an effective way of modelling variability in the relationship of UV-B-absorbing compounds to UV-B exposure. In contrast, statistical support for population-level random effects would provide evidence of local-scale interactions mediating the UV-B response within different populations.

Support for the different model types was evaluated in a number of ways. Model performance was compared using the constrained Akaike Information Criterion (AICc), and likelihood ratio tests were used to confirm the inclusion of the random-effects structures compared to models with no random-effects structures following the procedure noted in Zuur et al. (2009). We checked the residuals of the overall best models selected according to these criteria to test for evidence of spatial autocorrelation, and for other trends in residuals to confirm appropriate model fit. AICc weights were also estimated from the AICc scores to provide additional evidence to evaluate the relative importance of different model types at different exposure-period lengths. Finally, since some authors have noted that the estimation of random effects might be biased when low numbers of sample groups are used, we re-ran the analysis with subgenus- and population-level variation incorporated as fixed effects. Here, an additive model would represent a similar underlying logic to a random intercept model, whilst an interactive model would represent a random-slope and intercept. We compared the fixed-effects models using AICc and also used likelihood-ratio tests (see Supporting Information 4).

All data analyses was conducted using R version 4.0.3 (2020-10-10) using NLME (Pinheiro et al., 2020, version 3.1–145), GGEFFECTS (Lüdecke, 2018) and packages within the tidyverse (Wickham et al., 2019). The raw data associated with this study are deposited in the Dryad Digital Repository (Seddon et al., 2021a), and the scripts to reproduce all analyses and figures are uploaded at Zenodo (Seddon et al. 2021b).

3 | RESULTS

Models with random effects specified at the population level always out-performed (i.e. had lower AICc than) models with random effects specified at the subgenus level. Models which incorporated population-level effects also compared favourably to models not incorporating any population-level interactions (Figure 2). Across all model specifications, the overall best model was a random intercept-only model using unweighted UV irradiance (AICc = −84.16) with an exposure period of 19 days (Figure 3), although the AICc scores varied very little across accumulation periods from 8 to 28 days (Figure 2). The value of the coefficient related to the overall fixed effect in the best model (i.e. an exposure period of 19 days) was significantly different from zero (0.047 ± 0.022, $p = 0.039$; Table 2). The result was similar when weighted UV-B irradiance was used as a predictor variable, although the fixed effect was not significantly different from zero (0.035 ± 0.02, $p = 0.081$) for the same exposure period (Table 2).

Results from the models allowing for both random intercept and slopes also showed a clear contrast in model performance between different exposure-period lengths. The optimum accumulation-period length was estimated to be 12–13 days for both weighted and unweighted-radiation dose (AICc = −82.76), with model performance much worse for very short (<10 days) and longer (>20 days)
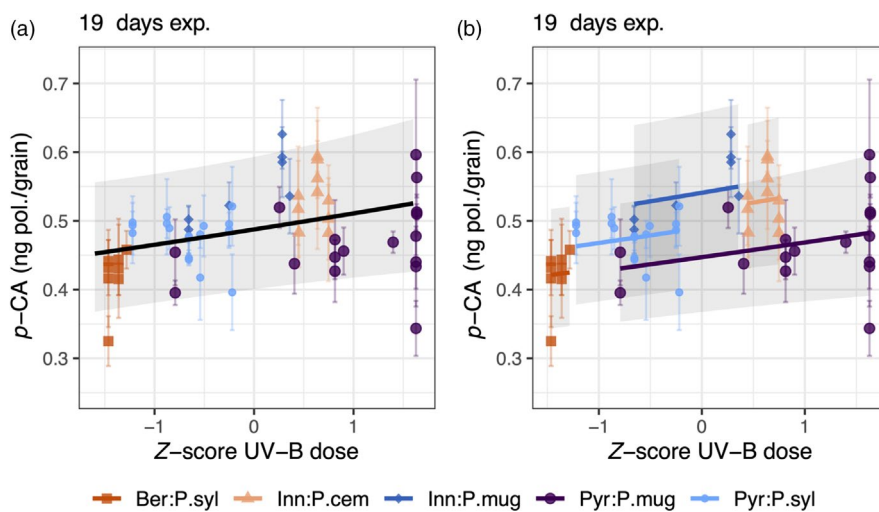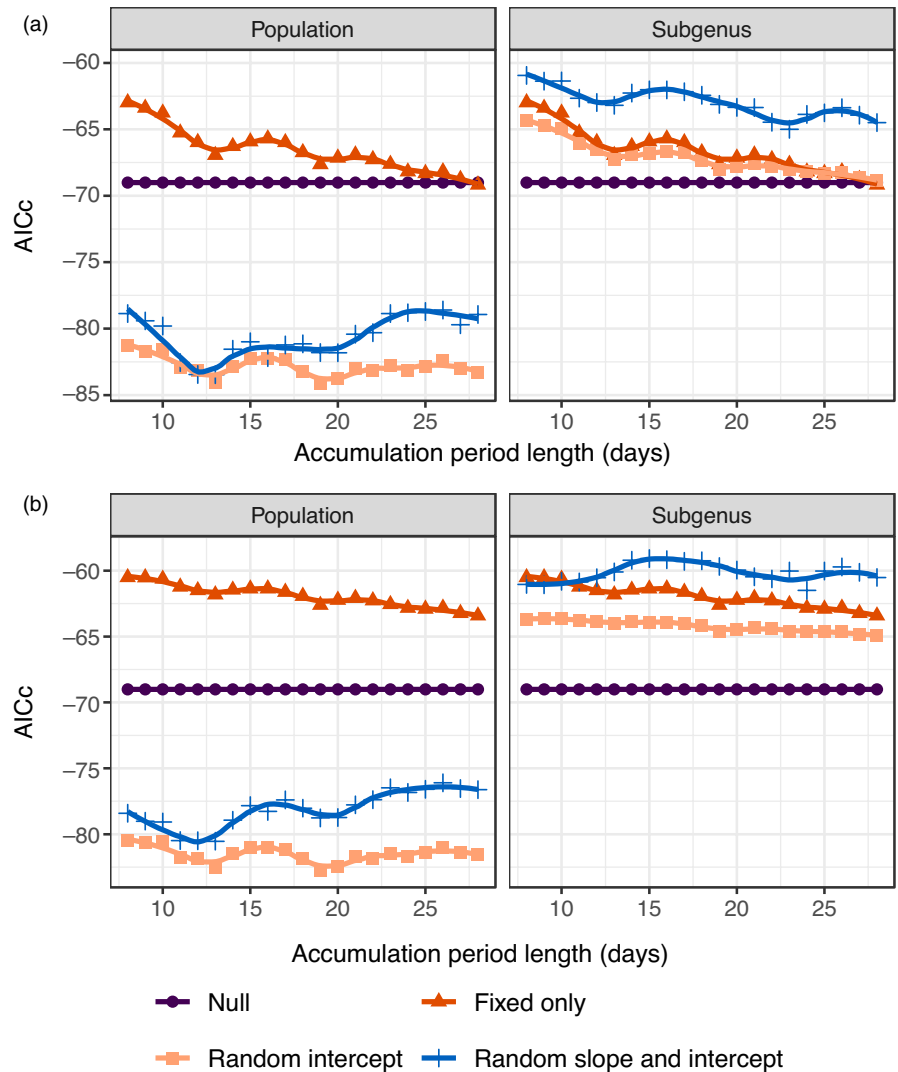
FIGURE 3 Content of *para*-coumaric acid in *Pinus* spp. pollen plotted against 19-day cumulative radiation doses (a) random-intercept intercept model for exposure to 19-days unweighted UV-B radiation. The dark black line and grey shading represents the predictions for the overall fixed effect in the model with the 95% prediction intervals. Panel (b) is the same as (a), but here the coloured lines represent the predictions once random effects are taken into account. The dark black line and grey shading represents the predictions for the overall fixed effect in the model with the 95% prediction intervals

TABLE 2 Fixed-effects model coefficients for the best models selected for both weighted and unweighted UV-B exposure

| Exposure period	Fixed term	Random effects structure	Coefficient	Value	Standard error	t value	p value
13	Unweighted UV-B	Slopes and intercept	Intercept	−0.712	0.044	−16.2	0.0000
13	Unweighted UV-B	Slopes and intercept	Unweighted UV-B	0.074	0.041	1.8	0.0763
13	Weighted UV-B	Slopes and intercept	Intercept	−0.725	0.053	−13.6	0.0000
13	Weighted UV-B	Slopes and intercept	Weighted UV-B	0.038	0.034	1.1	0.2713
19	Unweighted UV-B	Intercept only	Intercept	−0.718	0.042	−17.3	0.0000
19	Unweighted UV-B	Intercept only	Unweighted UV-B	0.047	0.022	2.1	0.0386
19	Weighted UV-B	Intercept only	Intercept	−0.720	0.049	−14.6	0.0000
19	Weighted UV-B	Intercept only	Weighted UV-B	0.035	0.020	1.8	0.0806

exposure periods. Here the confidence of the fixed effect of the optimal model was lower than when only random intercepts were used, since the fixed effect was not significant at $p = 0.05$ (Table 2). The reason for the reduced confidence in the fixed effect of the random slopes and intercept model is likely the result of the variable slopes and intercepts estimated between the different populations (Figure 4). For example, for the model estimated for a 13-day accumulation period, all populations showed positive relationships with UV-B radiation apart from *Pinus sylvestris* sampled from the Pyrenees.

Although AICc weights suggested overall equal support for the intercept only or random-intercept only model at this time period (Figure 5), likelihood-ratio tests did not indicate support for inclusion of both random slopes and intercepts (Table 3). Taken together, there was strongest support for a model which incorporated a population-level random intercept using our mixed effects modelling approach.

The general patterns of the results, identified using random-effect models, were replicated when categorical variables were included as fixed effects. Overall, the analysis using fixed effects also indicated that accounting for population-level variation allows for more of the variability in the UV-B-absorbing compound *para*-coumaric acid to be explained. However, among fixed-effects models, inclusion of a population-level interaction term was optimal according to AICc (between 12 and 13 days). For the random-effects models, likelihood-ratio tests supported the inclusion of this more complex interaction term, although confidence in the coefficient estimates for the within-population interactions remains low since none of these individual terms were significantly different from zero at $p = 0.05$ (see Supporting Information 4 for more details).

4 | DISCUSSION

Our study used *Pinus* spp. individuals, located along elevation transects from three countries across Europe, to investigate the relationship between pollen-chemistry composition and UV-B radiation. Our main results can be summarised as follows: (a) population-level variations were an important factor to consider when explaining the relationship of *para*-coumaric acid to UV-B radiation, because the

(a) **13 days exp.**
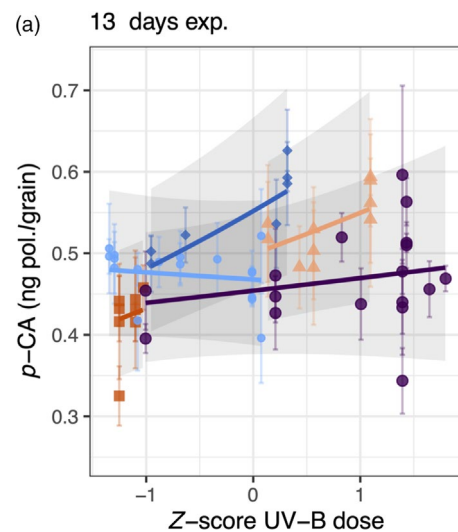
FIGURE 4 Content of *para*-coumaric acid in *Pinus* spp. pollen plotted against 13-day cumulative radiation doses. The fitted lines are the result of random-intercept model allowing for both random intercepts and random slopes. The grey shading represents the 95% prediction intervals. The colours representing the different populations are the same as in Figure 3

models which only incorporated random effects at the sub-genus level were comparable to, or performed worse than, models without random effects; (b) across all model types, there was strong statistical support for models allowing for varying intercepts using an exposure period of 19 days, which gave an overall positive relationship between the accumulation of UV-B-absorbing compounds and UV-B radiation along elevation transects; (c) there is also some statistical support for a model allowing varying slopes and intercepts, with an optimal exposure period of 11–13 days (Figure 5). Statistical support for this type of model specification is strengthened when a fixed-effects, rather than a random-effects, structure is used (Supporting Information 4). Below, we discuss the possible explanations of these findings and their implications for the development of a proxy for UV-B radiation based on the chemical variations found within *Pinus* spp. pollen grains.

FIGURE 5 Akaike Information Criterion weights plotted against different UV-B exposure times at the population or subgenus level. (a) Exposure to unweighted UV-B radiation. (b) Exposure to UV-B radiation weighted by the plant-growth action spectrum
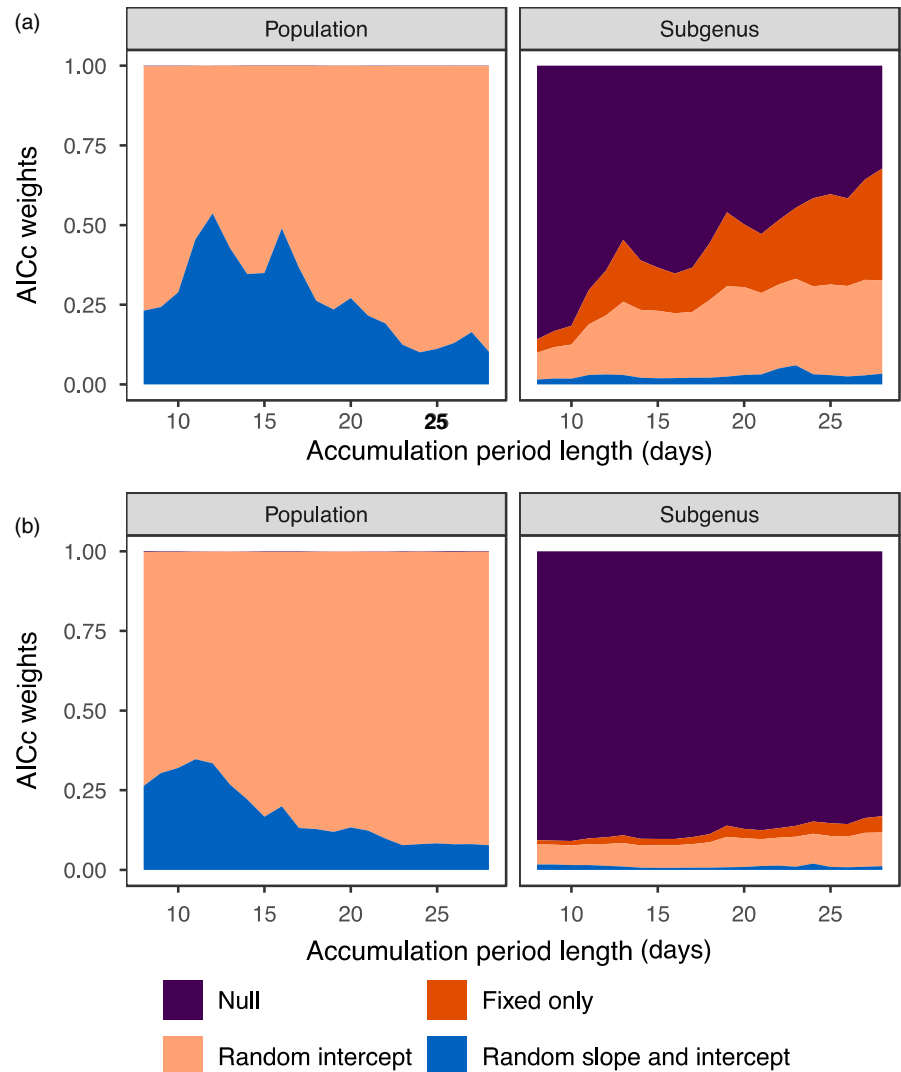
4.1 | The relationship between UV-B exposure and *para*-coumaric acid

The UV-B screening potential of *para*-coumaric acid (Fraser et al., 2014) in plants has been well established. Although this compound can act as precursor to a number of other phenolic compounds in the context of plant growth and defence, the presence of *para*-coumaric acid in pollen grains is thought to act to protect genetic material from exposure to damaging UV-B radiation (e.g. Li et al., 2019; Rozema et al., 2001). This is the same function thought to be relevant within plant epidermal cells (Fischbach et al., 1999; Kaffarnik et al., 2006), and it is assumed that the production of *para*-coumaric acid and other similar phenolic compounds was an essential factor in enabling the migration of early plants onto land (Weng et al., 2012).

Overall, there was strong statistical support for a model which enabled population-level interactions through a varying intercept when attempting to describe the relationship between UV-B exposure and *para*-coumaric acid. In this model, the overall coefficient to describe the relationship between *para*-coumaric acid accumulation and unweighted-UV-B radiation was positive and significant at $p = 0.05$,

although the effect size was low relative to the standard error. Even when the slopes and intercepts of the models were allowed to vary at the population level, four out of five populations demonstrated positive relationships between *para*-coumaric acid and UV-B radiation (Figure 3). Across our analysis, we also found that unweighted-UV-B irradiance provided a slightly better fit to the models than UV-B irradiance weighted by the plant-growth action spectrum, although in reality the type of action spectrum had a minimal effect in this study and so the minor differences in these results are not considered further here.

Thus, our data generally support the hypothesis that *para*-coumaric acid is correlated with UV-B radiation in field settings. These findings also agree with previous results both from other latitudinal/altitudinal correlation studies (e.g. Bell et al., 2018; Jardine et al., 2016; Lomax et al., 2008, 2012; Willis et al., 2011), as well as temporal calibrations-through-time (Jardine et al., 2016, 2020), and laboratory-based studies (e.g. Rozema et al., 2001). However, the fact that population-level models were identified as being better than the sub-genus models means that further considerations of causation are required before pollen-chemical variations can be used as a tool to reconstruct UV-B radiation in the fossil record.

TABLE 3 Likelihood-ratio tests for the two best UV-B accumulation periods selected for both weighted and unweighted UV-B exposure

Exposure period	Variable	Model structure	Model	df	AIC	BIC	logLik	Test	L. ratio	p-value
13	Unweighted UV-B	No random effects	1	3	−67.38	−61.25	36.69		NA	NA
13	Unweighted UV-B	Random intercept only	2	4	−84.79	−76.62	46.40	1 versus 2	19.41	5.27×10^{-6}
13	Unweighted UV-B	Random slope and intercept	3	6	−85.08	−72.82	48.54	2 versus 3	4.29	7.76×10^{-2}
13	Weighted UV-B	No random effects	1	3	−62.26	−56.13	34.13		NA	NA
13	Weighted UV-B	Random intercept only	2	4	−83.28	−75.11	45.64	1 versus 2	23.03	7.99×10^{-7}
13	Weighted UV-B	Random slope and intercept	3	6	−82.15	−69.89	47.07	2 versus 3	2.86	1.65×10^{-1}
19	Unweighted UV-B	No random effects	1	3	−68.07	−61.94	37.04		NA	NA
19	Unweighted UV-B	Random intercept only	2	4	−84.90	−76.73	46.45	1 versus 2	18.83	7.14×10^{-6}
19	Unweighted UV-B	Random slope and intercept	3	6	−83.42	−71.16	47.71	2 versus 3	2.51	1.99×10^{-1}
19	Weighted UV-B	No random effects	1	3	−63.05	−56.92	34.53		NA	NA
19	Weighted UV-B	Random intercept only	2	4	−83.50	−75.32	45.75	1 versus 2	22.44	1.08×10^{-6}
19	Weighted UV-B	Random slope and intercept	3	6	−80.38	−68.12	46.19	2 versus 3	0.88	4.96×10^{-1}

4.2 | Variations in the accumulation of UV-B absorbing compounds at the population level

The finding that population-level random effects were identified as the optimal models to describe variations in the response of UV-B-absorbing compounds in pollen was consistent irrespective of the UV-B exposure length, biologically effective spectral-weighting function, and the type of model structure used. One explanation is that the population-level variations are a result of local adaptation, whereby selection pressure may modify the response rate of the different taxa across their range (Benito-Garzón et al., 2011). Evidence for local adaptation has been identified in numerous tree species, including *Pinus sylvestris* (Rehfeldt et al., 2002; Savolainen et al., 2007). Although studies on local adaptation on *Pinus* spp. are generally focused on survival- and growth-related traits, particularly with respect to temperature, our study shows population-level variations in *Pinus* spp. pollen chemistry in response to UV-B radiation. This inference is supported by other studies into the chemical variations of pollen, and their links to responses to UV-B radiation and other variables (Bell et al., 2018; Diehn et al., 2018).

A second explanation is that the population-level random-effect structures may be explained by the result of interactions of UV-B radiation with other environmental variables. Both experimental (Coffey et al., 2017) and field evidence (Martz et al., 2007; Robson & Aphalo, 2019; Solanki et al., 2019) indicate that plants can screen UV radiation more effectively under low temperatures, and that the accumulation of UV-B-absorbing compounds in plant-leaf tissue can sometimes be the result of interactive effects with temperature at high elevations and latitudes (Neale et al., 2021). For example, Martz et al. (2007) assessed the variations in both the concentration and composition of UV-B-absorbing compounds in *Pinus sylvestris* needles using UV-exclusion field chambers in Finland, and found UV-A/B exclusion had a significant effect on five out of 46 soluble phenolic compounds within the leaf needles. However, they also showed that the effects of UV-B radiation on pigments, related to protection against light-induced oxidative stress, were only detectable under freezing temperatures and high solar irradiance in the spring. Indeed, because warmer temperatures can obscure the effects of UV-B radiation on leaf chemistry (e.g. through the leaf-flavonoid content, Coffey et al., 2017), it has been proposed that differences in the content of UV-B-absorbing compounds may derive from the interaction of temperature fluctuations and seasonal snow cover rather than UV-B exposure alone (Solanki et al., 2019).

We do not present evidence that the same mechanisms are driving the population-level differences identified in our results. However, these examples help to indicate the complexities related to the accumulation of UV-B-absorbing compounds in pollen at the site or population level. Until we know more about the environmental and genetic factors regulating the production of UV-B-absorbing compounds and their subsequent incorporation into the pollen grain, distinct population-level effects may be required to account for the variation of phenolic compounds in studies involving individuals sampled across large spatial gradients. Physiological studies into the

mechanisms that lead to the accumulation of UV-B-absorbing compounds within pollen, and improve our understanding of the interaction of UV-B radiation with other environmental variables therefore represent an essential avenue of future research.

4.3 | The timing of UV-B exposure on pollen development

Although the results of the optimal model varied depending on the random-effects structure, in general, with both weighted and unweighted UV-B radiation, models over intermediate UV-B exposure periods performed better than those over short (<11 days) or longer (>20 days) durations. A 19-day duration gave the best overall model, using an unweighted UV-B dose with random intercepts. A number of other studies using different environmental factors to explain variation in pollen chemistry also identify a similar period of exposure during pollen development. For example, protein and lipid content in pollen grains across a number of phylogenetic groups are best explained by temperature and precipitation variations over the 2-week period prior to pollen release (Zimmermann & Kohler, 2014). Similarly, Jokerud (2017) demonstrated that *para*-coumaric acid accumulates in pollen in greater quantities in male strobili from *P. sylvestris* sampled from branches which were shaded compared to branches which were unshaded only 1 month prior to pollen dehiscence.

Indeed, current understanding of the formation and maturation of pollen in the context of the *Pinus* reproductive cycle (Owens, 2006), indicates that the microspores, which eventually develop into individual pollen grains, are coated with their main sporopollenin component following the degeneration of tapetal cells within the *Pinus* male inflorescence (e.g. Dickinson & Bell, 1972; Jokerud, 2017; Rowley et al., 2000). Although the initial development of pollen in Pinaceae begins towards the end of the growing season in the autumn (Owens, 2006), the main stages of pollen development in Pinaceae occurs during the following spring (Lü et al., 2003). The crucial process in which the sporopollenin component develops occurs within an approximately 2-week period towards the end of pollen development prior to dehiscence (Owens, 2006). Our study is congruous with this finding, since across our models we found that the optimal duration for explaining the accumulation of *para*-coumaric acid in natural populations was between 12 and 19 days.

4.4 | Implications for fossil reconstructions

Taken together, these results reveal several insights in relation to the experimental design and interpretation of pollen-grain chemistry variations in fossil sediments used as a proxy for UV-B radiation. The overall importance of population-level interactions suggests that a single, continental-scale response function, which comprises of responses across multiple populations and latitudes (i.e. a model which incorporates no population-level effects), is difficult to derive

from this specific dataset. Since a single pollen type extracted from a sediment core could be representative of multiple populations or species, our findings indicate that population-level variation may be an important confounding effect when developing reconstructions of UV-B radiation based on pollen chemistry.

However, the development of a proxy for UV-B radiation based on pollen chemistry compounds should not be discounted, especially if factors related to the deposition, preservation and representation of pollen within a lake sediment sample are taken into consideration. The pollen source area of a site (defined as the area from which a fixed percentage of the pollen sampled at a site is derived, Davis, 1963; Jacobson & Bradshaw, 1981; Oldfield, 1970; Prentice, 1985), can vary depending on basin shape, size and fluvial input. In small lakes or forest hollows (e.g. <10 m in diameter), it is generally assumed that the main source area of the pollen will be the surrounding 20–30 m (Jacobson & Bradshaw, 1981), with local vegetation overriding any signal from the regional vegetation. In contrast, a lake which is approximately 100 m in diameter will be represented by extra-local pollen (i.e. from plants growing 20 to several hundred metres from the lake), in addition to incorporating a larger regional component (Jacobson & Bradshaw, 1981; Sugita, 1994). Pollen representation is also influenced by taxon-specific factors related to differential dispersal and production (e.g. Prentice, 1985), in addition to the heterogeneity of the surrounding landscape (Hellman et al., 2009; Sugita, 1994), so that the representative source area of the lake can have a major impact on the design of the reconstruction study.

If, as our data suggest, local-population responses dominate over larger-scale continental responses, then palaeoecologists should shift their focus towards developing reconstruction models which are based on lake sites with smaller source areas. Since the contribution of local populations also declines from the centre of the lake, choosing reconstruction sites with smaller basin and source areas can minimise the potential input from extra-local populations, and thereby counteract the effect of smoothing of any pollen-chemistry signatures from larger areas. We suggest that a carefully considered study design should allow future research to minimise uncertainties surrounding our knowledge of the relationship between pollen chemistry and UV-B radiation at the population level, and the next step will be to determine how the relationship between UV-B radiation and pollen chemistry varies (both in terms of slopes and intercepts) across a larger set of representative populations. Since sedimentation rates also vary both within and between cores (Holocene sediment accumulation rates globally vary between 0.05 and 0.07 cm/year, Jenny et al., 2019), a typical Holocene sediment sample integrates variation in UV-B radiation over ~7–10 years. This means that reconstruction models based on sample analysis over multiple years may also be necessary to establish how the within-population responses scale up across multiple years.

After consideration of the practical issues related to the calibration-model design, a logical next question involves the practicalities of the application of pollen-chemistry methods to

sediment sequences. First, analysis of pollen-chemistry compounds requires individual pollen grains to be extracted from the sediments. However, whilst this may be time consuming, a number of methods already exist that can help to isolate pollen grains from sediments, including extraction using a pipette (Seddon et al., 2017), flotation using density separation (Eriksson et al., 1996) and flow-cytometry techniques (e.g. Tennant et al., 2013). Thus, we do not consider this issue to be of a major hindrance for assessing chemical variations in fossil sequences and there have already been three studies which have successfully extracted and reconstructed chemical variations from the phenolic compounds preserved within fossil-pollen grains (Jardine et al., 2016, 2020; Willis et al., 2011). A second, related issue, will be to develop methods to generate closer analogues between fossil- and modern-pollen samples. Chemical analyses of fossil-pollen grains indicate that phenolic compounds occur as bound components within the pollen-grain exines (Li et al., 2019), but may also exist in other parts of the pollen grain (e.g. as free compounds within the pollen cytoplasm, Rozema et al., 2001). There are a number of procedures proposed in the literature for extracting the desired sporopollenin-based compounds in pollen-grain exines, including: saponification (Nierop et al., 2019); acetolysis (Jardine et al., 2015); and methanol extraction (Rozema et al., 2001), but further work is required to investigate their relative effectiveness and reproducibility for targeting the sporopollenin-based components (e.g. Jardine et al., 2017). We suggest that the creation of better modern analogues, in addition to resolving the response functions of phenolic compounds to UV-B radiation across multiple populations, remain key research priorities moving forward.

5 | CONCLUSIONS

We investigated the response of the phenolic compound para-coumaric acid in four taxa belonging to the genus *Pinus* in relation to variations in received UV-B radiation. We show that there is an overall positive relationship between variation in para-coumaric acid from fossil pollen and UV-B radiation, which is best described by using an intermediate UV-B-exposure period between 12 and 19 days prior to pollen release. However, this relationship is modified by site-specific factors, implying that researchers should be cautious about developing calibration models based on pollen-chemistry variations without the specific site context. In addition, our results suggest that, although pollen-based chemical reconstructions in the palaeoecological record may still be possible, their assembly requires careful assessment of the probable factors influencing the pollen deposition at a site. Future improvements are dependent on increased mechanistic understanding of the local factors which can mediate the UV-B response in pollen grains across different populations, and in upscaling knowledge of responses at the plant level to the longer-term variations represented within a sediment-core sample.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

A.W.R.S., D.F. and T.M.R. planned and designed the research; A.W.R.S., D.F., M.N., T.M.R., R.G. and S.A.H.Ö. conducted fieldwork; A.W.R.S., B.H. carried out the radiation modelling; A.W.R.S., M.N., L.C.K. and S.A.H.Ö. prepared and analysed the samples for py-GC-MS; A.W.R.S. analysed the data, with contributions from D.F., M.N., BH and T.M.R.; A.W.R.S., D.F., M.N., T.M.R. and B.H. wrote the manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The raw data associated with this study are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.4j0zpc8bk (Seddon et al., 2021a), and the scripts to reproduce all analyses and figures are uploaded at Zenodo http://doi.org/10.5281/zenodo.4896732 (Seddon et al., 2021b).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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