Methodology

Coring and sub-sampling

A 46-cm-long core was collected from the centre of a mire at 1000 m a.s.l. on Galdhøpiggen, Jotunheimen. A Russian peat corer with a 5 cm diameter was used for the extraction of the core. Whilst the peat layer of the mire extended deeper, only the top 46 cm (including the surface moss polster) was extracted and analysed for the purposes of this study. The core was wrapped in plastic film and placed in a plastic tube for transportation to Swansea University's cold store. The core was then stored in dark conditions at 5 °C prior to sub-sampling.

For laboratory analyses, the core was sub-sampled at 1 cm intervals to produce a total of 46 samples. A modified syringe was used to measure out a known volume (1 cm^3) of peat material which was then placed in a labelled plastic test tube. The 5 cm thick moss polster covering the top of the core was removed and cut into segments at 1 cm resolution from the top down. To assure enough pollen was extracted from each segment (i.e., 'depth'), 2 cm^3 of material was measured with the modified syringe and placed in a test tube. Prior to analysis, 2 tablets of known concentration of an exotic marker spore (*Lycopodium*) and 20 ml of water were added into the test tubes containing the peat- and the moss polster samples.

Extraction of pollen from peat

The full extraction procedure is outlined by Bennett and Willis (2001). What follows here is the protocol that was used for the analysis of the peat core, with some modifications from Bennett and Willis. Whilst the use of hydrofluoric acid (HF) is common in the extraction process to remove silica and silicates, the mineral content of the peat core used in this study was minimal as it consisted largely of organic material. A number of samples were tested prior to analysis to determine whether HF was required to produce clear slides where pollen is easily distinguishable under the microscope. Four samples were treated with HF, whilst another four samples followed the rest of the procedure but skipped the HF step. Comparison of the output from both types of samples established that both approaches produced equally good results and it was decided to discontinue the use of the HF extraction.

Step 1: To estimate the concentration of the pollen and spores, a known volume of exotic suspension was added to each sample (Stockmarr, 1971). Two tablets of *Lycopodium* (Batch no. 3862, concentration: 9666 spores per tablet) were added to each test tube containing 1 cm^3 of peat sample and 20 ml of water. The contents were mixed using a whirlimix and left overnight to allow the tablets to dissolve.

Step 2: To remove carbonates, 7% HCl was added to each test tube, and tubes were placed in a hot water bath at 90 °C for 30 minutes. This step was followed by a water wash, i.e., samples were centrifuged at 3000 rpm for 5 minutes, excess liquid was decanted and remaining sample whirlimixed, distilled H_2O was added, and samples were centrifuged again. The water wash was repeated several times until the supernatant was clear.

Step 3: The removal of humic acids and the breakdown of sediment was achieved by adding 10% NaOH to the tubes and placing them in a water bath at 90 °C for 5 minutes.

Step 4: The mixture in each tube was then sieved through a fine sieve (180 µm mesh size) using distilled water to remove large particles. This step was followed by several consecutive water washes of the remaining fine material.

Step 5: Glacial acetic acid (CH_3COOH) was used as a deflocculant; samples were centrifuged and liquid decanted.

Step 6: The acetolysis step removes polysaccharides (e.g., cellulose), which are present on the surface of pollen grains and often in the sediment. Nine parts of acetic anhydride ($(CH_3CO)_2O$) were mixed with one part of sulphuric acid (H_2SO_4) and the mixture was added to the remaining material. The test tubes were then placed in a water bath at 90 °C for three minutes.

Step 7: Samples were washed with CH_3COOH and transferred into smaller tubes, followed by several water washes.

Step 8: To increase the contrast of pollen grains, three drops of aqueous safranin dye was added to each sample; samples were then washed with water.

Step 9: Tertiary butyl alcohol (TBA) was added to the samples to dehydrate the pollen grains and the remaining sample was transferred into small tubes. Silicone oil was added and thorougly mixed into the sample with a cocktail stick to avoid clumping of the material. The tubes were left open for 24 hours to let the TBA evaporate.

Step 10: Remaining residue from each sample was mounted on individual microscope slides, covered with a coverslip and sealed. Each slide thus obtained represents a given depth in the profile.

Pollen identification and counting

Microscope slides with residue were placed under a Leica DM 2000 light microscope and examined in transverse sections, using magnifications of x400 and x1000. All encountered pollen grains were counted and identified according to their unique shape, size, and surface pattern (Bennet and Willis, 2001). Reference material was used to compare the unidentified grains with pollen grains of known taxa. These materials ranged from reference slides containing real pollen grains (Swansea University collection), to published guides containing photographs and keys (e.g., Moore, 1994; Reille 1992), to online databases with detailed descriptions (e.g., *paldat.org*). Following the protocol, a total of 500 land pollen (TLP) grains and spores were counted and identified per depth level, excluding the added exotic.

Calculation of pollen relative abundance, concentration, and accumulation rates

Pollen relative abundance and concentration at each depth were calculated following Bennett and Willis (2001). The relative abundance of each taxon was calculated by dividing the pollen grain count of a taxon at a given depth with the sum of TLP counted at that depth:

$$Relative\ abundance\ (\%) = \frac{Counted\ pollen\ of\ a\ taxa}{Sum\ of total\ land\ pollen}$$

The known volume of added exotic (*Lycopodium*) was used to calculate the fossil pollen concentration (no. of grains per unit volume of peat, e.g., grains cm^{-3}) of each taxon by using the following equation:

$$Concentration = \frac{Counted\ pollen\ of\ a\ taxa}{Counted\ Lycopodium} \times \frac{Lycopodium\ spores\ per\ tablet\ \times\ no.\ of\ tablets}{Sediment\ volume\ (cc)}$$

If sediment accumulation rates are known, pollen concentration values can be used to calculate pollen accumulation rates, i.e., the number of pollen grains incorporated annually per unit area of substrate (grains cm^{-2} $year^{-1}$) :

$$Pollen\ accumulation\ rate\ (PAR) = \frac{pollen\ concentration\ (cm^3)}{sediment\ accumulation\ rate\ (cm\ yr^{-1})}$$

Radiocarbon dating and age calibrations

The humin fraction of three peat samples (taken at 10 cm, 24 cm and 46 cm depth) was dated using accelerator mass spectrometry (AMS) radiocarbon dating at Queen's University Belfast. The resulting ^{14}C dates for samples taken at depths of 24 cm and 46 cm were converted to calendar ages (cal. yr BP) using the calibration software CALIB (8.2) (Stuiver et al., 2021) and the IntCal20 radiocarbon age calibration curve (Reimer et al., 2020). The dates were determined using a 2σ error (Stuiver and Reimer, 1993) meaning that there is a 95% probability that the reported median calibrated age falls within the estimated age range. The 'greater than modern' radiocarbon date ($F^{14}C$ = 1.0915 ± 0.0021) at 10 cm depth was calibrated using the post-bomb calibration dataset 'Northern hemisphere zone 1' (NHZ1) by Hua et al. (2013) and the online calibration resource CALIBomb (Available from: calib.org/CALIBomb/). This dataset was selected because it is appropriate for latitudes in the northern hemisphere above ~40°N, which is the closest match to the Galdhøpiggen study site.

The R package 'clam' (2.2) (Blaauw, 2010) was then used to create an age-depth model based on linear interpolation between the three dated levels of the core (10, 24 and 26 cm) and the surface. The input file contained both ^{14}C ages and the calibrated $F^{14}C$ date. By default, 'clam' uses the northern hemisphere terrestrial calibration curve (IntCal13) from Reimer et al. (2013), and the calibration assumes a Gaussian distribution. The post-bomb calibration curve is again provided by Hua et al. (2013). To include the negative (i.e., post-1950 AD) radiocarbon date, the 'NHZ1' calibration curve was selected (R command: *postbomb=1*), and the calendar scale was set to BC/AD (R command: *BCAD=TRUE*). In the input *.csv* file, the ^{14}C ages were included for depths 24 cm and 46 cm. The non-^{14}C dates (i.e., at 10 cm and at the surface) were assigned negative values to indicate their departure from 1950 AD towards modern times.

Knowing that the calibrated date at 10 cm is c. 2000 AD, this depth was assigned the value '-50' in the input file. The surface of the consolidated peat core was set at 6 cm, excluding the modern moss polster. Assuming that the 5 cm thick moss polster represents 2-4 years of pollen accumulation (Lisitsyna and Hicks, 2014) and taking into account that the core was collected in 2018, the age of the surface was set to 2014 (represented by '-64' in the input file). An age-depth model was created in R (3.6.3). The calibrated output for ^{14}C dates was comparable to the age ranges produced by the CALIB (8.2) software (Stuiver et al., 2021).