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The fungal ecology of the Brassington Formation (Middle Miocene) of Derbyshire, United Kingdom, and a new method for palaeoclimate reconstruction

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Fossil fungi from periods warmer than modern climates provide unique insights into the future impacts of anthropogenic climate change. Here we report the fossil fungal assemblage from the late Middle Miocene Kenslow Member of central England, associated with climatic conditions warmer than the present-day. The identification of 110 morphotypes, which primarily relate to moist environments and the presence of wood, have been used to develop a new nearest living relative palaeoclimate reconstruction. The fungal assemblage indicates a Köppen–Geiger climate class, represented by temperate conditions, no dry season, and warm summers. This new fungal-based palaeoclimate reconstruction technique holds exciting potential to explore critically important but poorly understood palaeoenvironments, and the resulting qualitative inferences align well with previously published palaeobotanical quantitative estimates of palaeoclimate. These findings show that diverse fungal assemblages can successfully be used to reconstruct past climates for the first time.

KEYWORDS

wetlands, fungi, biodiversity, nearest living relative, palynology, climate change

Introduction

A lack of long-term and large-scale information on how fungi will respond to climate change prevents determining how ecological and historical factors potentially affect the evolution in space and time of fungal assemblages in different regions (Classen et al., 2015; Willis, 2018). Fungal biogeography has been shown to be controlled by climate,

with mycorrhizal taxa having the narrowest climate tolerances (Větrovský et al., 2019). Intriguingly, Větrovský et al. (2019) found that overall fungal diversity was highest in the high northern latitudes, whereas coprophilous fungi and saprotrophs typically exhibit maximum taxonomic richness in the tropics (Richardson, 2001; Hyde et al., 2016). This latitudinal diversity gradient in fungi has also been observed during the Miocene Climatic Optimum [MCO – 17.5–14.5 million years ago (Ma)] (Romero et al., 2021). Observations during the late 20th Century have shown that soil fungal diversity is highly variable, with studies indicating both increased and decreased diversity in relation to warmer temperatures (Melillo et al., 2017; Delgado-Baquerizo et al., 2020; Steidinger et al., 2020). In light of the role fungi play in many ecosystem services, such as carbon sequestration, the lack of information on how warmer than modern climates will modify fungal assemblage composition, distribution and ecology becomes a pressing knowledge gap (Willis, 2018). The presence of fossil fungi in sedimentary successions deposited during warmer-than-modern deep time intervals provides an opportunity to fill this knowledge deficit and inform new predictions of the future response of fungi to warming from anthropogenically driven climate change.

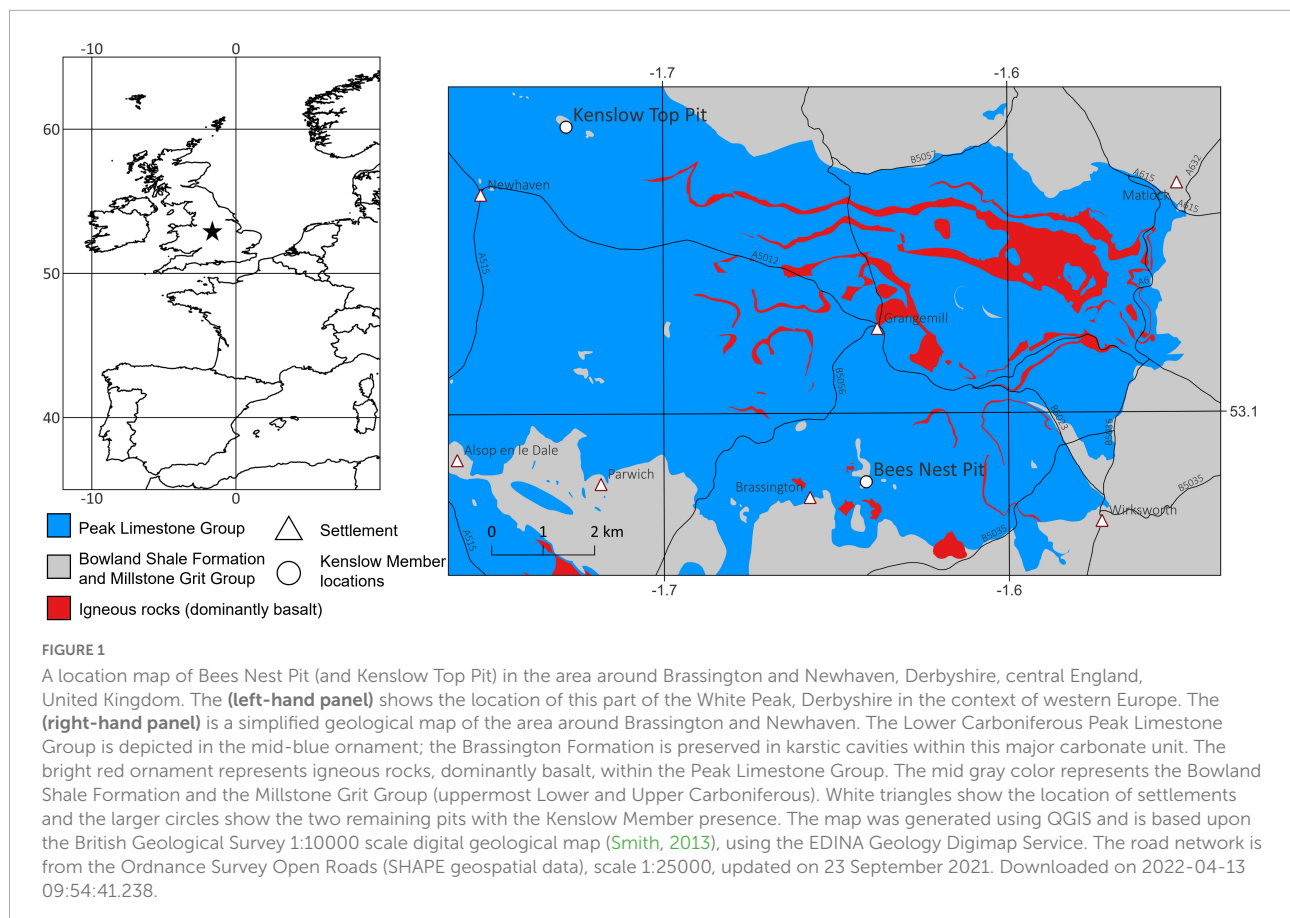
The Miocene epoch (23.03–5.33 Ma) was an interval of geological time that was predominantly warmer than present (Pound et al., 2012a; Holbourn et al., 2018; Steinthorsdottir et al., 2021). The warmest interval of the Miocene was the MCO, during which diverse forests reached the Arctic Circle (Pound et al., 2012a; Steinthorsdottir et al., 2021) and modeled global air temperatures were 5°C warmer-than-present (Henrot et al., 2017; Burls et al., 2021). Following this climatic optimum, there was a progressive cooling toward near-modern sea surface temperatures by around 7 Ma (Herbert et al., 2016). However, this cooling is not recorded in the mid-latitudes of the North Atlantic region, where temperature gradients appear flat and indicate the presence of the warm oceanic current (Super et al., 2020). This warm current was likely responsible for the relatively warm temperatures observed in terrestrial palaeobotanical sites in western Europe (Utescher et al., 2015; Gibson et al., 2022; McCoy et al., in review). Evidence for Miocene biodiversity, climate, and environments in the United Kingdom (UK) are limited to isolated and poorly dated deposits, of which the Brassington Formation of central England is the most extensive and best dated (Walsh et al., 1987, 1996; Wang et al., 2018; Pound et al., 2012b; Pound and Riding, 2016). The Brassington Formation is present in around 60 karstic hollows and consists of three members: Kirkham, Bees Nest and the uppermost Kenslow Member. Only the uppermost unit, the Kenslow Member, is fossiliferous (Boulter and Chaloner, 1970; Pound et al., 2012b, 2019; Pound and Riding, 2016; Walsh et al., 2018; O'Keefe et al., 2020; McCoy et al., in review). The Kenslow member, the focus of this study, is composed of around 6 m of massive grey clay with common plant fragments near the top (Boulter et al., 1971; Walsh et al., 2018). The palaeoenvironment

of the Kenslow Member has been interpreted to be a pre-subsidence lake, e.g., a cut-off lake on the Miocene flood plain, a post-subsidence hollow similar to sinkhole lakes or a wetland (Walsh et al., 1972; Pound et al., 2012b; McCoy et al., in review; respectively).

The Kenslow Member, dated to the late-Middle to Late Miocene (Serravallian-Tortonian), has yielded diverse fossil pollen, spore, and palaeobotanical remains that represent a subtropical to warm-temperate mixed forest (Boulter and Chaloner, 1970; Pound et al., 2012b; Pound and Riding, 2016). Climate reconstructions based on the co-existence approach indicate a Mean Annual Temperature (MAT) of 17–18.4°C, with mild winters of > 6.2°C and hot summers of 26.5–28.3°C (Pound and Riding, 2016). However, Gibson et al. (2022) proposed a cooler reconstruction using statistical techniques. In their reconstructions, MAT was reconstructed as 13.7°C ± 0.3°C, with winters of 4.3°C ± 0.8°C and warm summers of 21.9°C ± 0.3°C (Gibson et al., 2022). Both studies proposed higher than modern Mean Annual Precipitation (MAP) of 1096–1562 mm (Pound and Riding, 2016) and 955 mm ± 150 mm (Gibson et al., 2022). However, Pound and Riding (2016) proposed a degree of precipitation seasonality in the Serravallian of the United Kingdom, whereas Gibson et al. (2022) reconstructed a low degree of seasonality. Recent research has provided the first evidence of fossil fungal remains (Pound et al., 2019), and continued study revealed an abundant and diverse fungal assemblage. Given the uncertainty in how modern fungi will respond to anthropogenically driven climate change, the fungal assemblage in the Kenslow Member provides evidence for the diversity of the Dikarya group under a warmer and wetter climate than present-day. The aim of this paper is to document the biodiversity and ecology of the fungi preserved in the Kenslow Member and to provide evidence for how these assemblages change within a developing wetland (McCoy et al., in review) during warmer than present conditions. The fossil fungal assemblage is also used to reconstruct qualitative climate information and this is compared back to previous palaeobotanical reconstructions.

Materials and methods

Samples were collected from the Kenslow Member at Bees Nest Pit (NGR SK 1818 6158; 53.09°N; 1.64°W), a disused sand quarry ca. 1 km northeast of Brassington village, near Wirksworth, Derbyshire, United Kingdom (Figure 1). Approximately 50 g of clay were collected from each of the 17 sampling points along a 2.30 m vertical transect that covered the entire exposed Kenslow Member (Figure 2). Samples came from the blue-gray clay and were spread across the entire Kenslow Member, but with concentrations at woody horizons (Figure 2). To maximise the diversity of fungi recovered, additional samples of clay were taken from the



cracks in fossil wood recovered from the site. At least 5 g of each sample were prepared using either an acid digestion methodology (following Pound and Riding, 2016; Pound et al., 2021; Riding, 2021) or a non-acid density separation technique (following O'Keefe and Eble, 2012; Caffrey and Horn, 2013). Samples were crushed to < 1 mm prior to processing and sieved through a 250-micrometre top sieve, and where non-acid methods were utilized, a 5-micrometre bottom filter was used to facilitate removal of the remaining clay. Samples were analysed using Leica DM750 and DM2000 microscopes and photographed with Leica ICC50W cameras run by Leica Application Suite® software at both Morehead State University, United States and Northumbria University, United Kingdom. Entire slides were analysed for fungal palynomorphs to ensure maximum identification of taxa (presence) for the palaeoclimate reconstructions technique. NLR techniques rely primarily on presence/absence of taxa, when applied to dispersed remains (Utescher et al., 2014). For this reason and because the number of fungal individuals produced vs. preserved are very poorly understood (Taylor et al., 2015), numbers of individual fungal morphotypes were not counted for this study, rather, morphotype presence-absence was recorded. Z-stacked images were constructed to resolve fine details using Helicon Focus®. Slides prefixed with MPA are archived at

the British Geological Survey. Slides with a Wood Sample designation are housed at the Palynology Lab of the Cold and Palaeoenvironments Research Group at Northumbria University. Slides with a numerical-only designation are housed at the OPaL Lab at Morehead State University (**Supplementary Table 1**).

Fossil fungi were first grouped following Nuñez Otaño et al. (2021) and Romero et al. (2021). Taxonomic identification, when possible, was completed using morphometric methods through comparisons with accepted species^{1,2} based on the primary literature of taxa encountered (Ellis, 1971, 1976; Ellis and Ellis, 1997, 1998; Seifert et al., 2011; Guarro et al., 2012; among many others – see **Supplementary Table 2**), comparisons with material held in reference collections at Morehead State University and CICyTTP-Prov. ER-CONICET-UADER, Argentina, and those recorded in the John Williams Index of Palaeopalynology (JWIP) (Riding et al., 2012), and the Non-Pollen Palynomorph Image Database (NPP-ID³) (Shumilovskikh et al., 2022). Fungal remains that

1 www.indexfungorum.org

2 www.mycobank.org

3 <http://non-pollen-palynomorphs.uni-goettingen.de/>

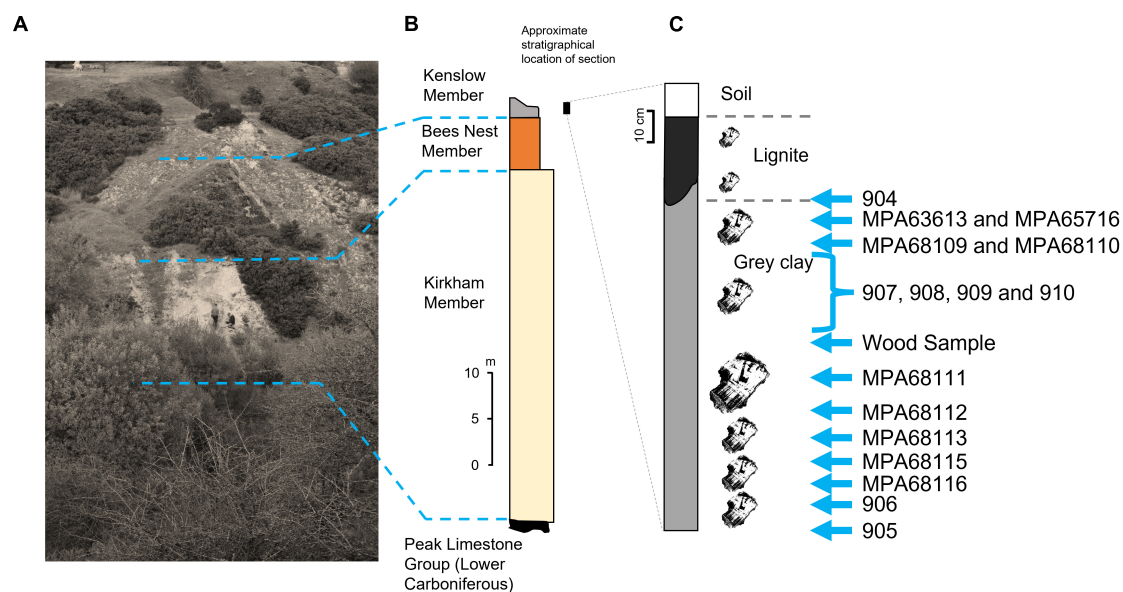


FIGURE 2

The lithostratigraphy of the Brassington Formation and the samples from the Kenslow Member analysed herein. (A) A field photograph of the exposed section at the central north part of Bees Nest Pit, note people for scale. The dashed lines indicate the boundaries between the three members in this succession, which is the type section of the Brassington Formation. (B) A generalised lithostratigraphy of the Brassington Formation at Bees Nest Pit based on Boulter et al. (1971) and O'Keefe et al. (2020), note the black bar to show the relative location and scale of the section in (C) to (B). (C) A simplified lithological log of the Kenslow Member at Bees Nest Pit. Wood silhouettes indicate the position of fossil wood horizons (the wood fragment sizes reflect the sizes observed in this section), and blue arrows indicate the location of the samples used in this study.

were not able to be identified were assigned an identifying acronym following O'Keefe et al. (2021); in this case, the acronym BN for Bees Nest and a number were used (e.g., BN-31). Full descriptions of the BN taxa are available in [Supplementary Table 1](#).

Fungal morphological and functional traits along with their geographical distributions were determined through comparison with the primary literature ([Supplementary Table 2](#)) and FungalTraits v. 1.2 database (Pölme et al., 2020) using the Nearest Living Relative (NLR) approach. To reconstruct Miocene climate, a simple NLR approach was applied. Once the Kenslow Member fossils were identified, a literature and online database survey was conducted until May 2022 in order to identify known global occurrences of each taxon (full reference list available in [Supplementary Table 2](#); Pound et al., 2022).

After application of the Sorensen Index to the presence-absence data of fungal palynomorphs, a non-metric multidimensional scaling (nMDS) and group cluster analysis were used to ordinate the samples. The Sorensen Index was chosen because sample sizes were adequate and it enables the use of presence-absence data (Schneck and Melo, 2010). The cluster analysis was run with a similarity profile (SIMPROF) test, with 9999 permutations, to identify groups of samples based on their fungal similarities. SIMPROF seeks to identify clusters within a hierarchical cluster analysis whereby no further branching can be identified with increasing similarity

and groups can be considered homologous (Clarke et al., 2008). An Analysis of Similarity (ANOSIM) test was used to explore whether sample type (wood versus sediment) or processing method (acid digestion versus non-acid technique) had influenced the results. All analyses were performed in Primer 6 (Plymouth Routines in Multivariate Ecological Research version 6, PRIMER-E Ltd., Plymouth Marine Laboratory, United Kingdom) following a presence/absence pre-treatment in the same software (Clarke and Warwick, 2001; Clarke and Gorley, 2006).

To reconstruct palaeoclimates from fungi, a NLR approach was applied. This branch of palaeoclimate reconstruction techniques builds on the conceptual framework of the present being analogous to the past and is routinely applied to paleobotanical remains (e.g., Utescher et al., 2014; Gibson et al., 2022). For paleobotanical applications, the present-day distribution of a fossil's NLR is used to derive climatological values. These present-day climate values of each fossil taxon in an assemblage are then analysed in various ways to produce numerical palaeoclimate values (Thompson et al., 2012; Utescher et al., 2014; Gibson et al., 2022). In developing a method to apply to fungal remains, it became apparent that the known present-day distribution of many fungi are not as well documented as plants. To circumvent this limitation, we did not reconstruct numerical values and instead focused on a qualitative reconstruction through the Köppen–Geiger system

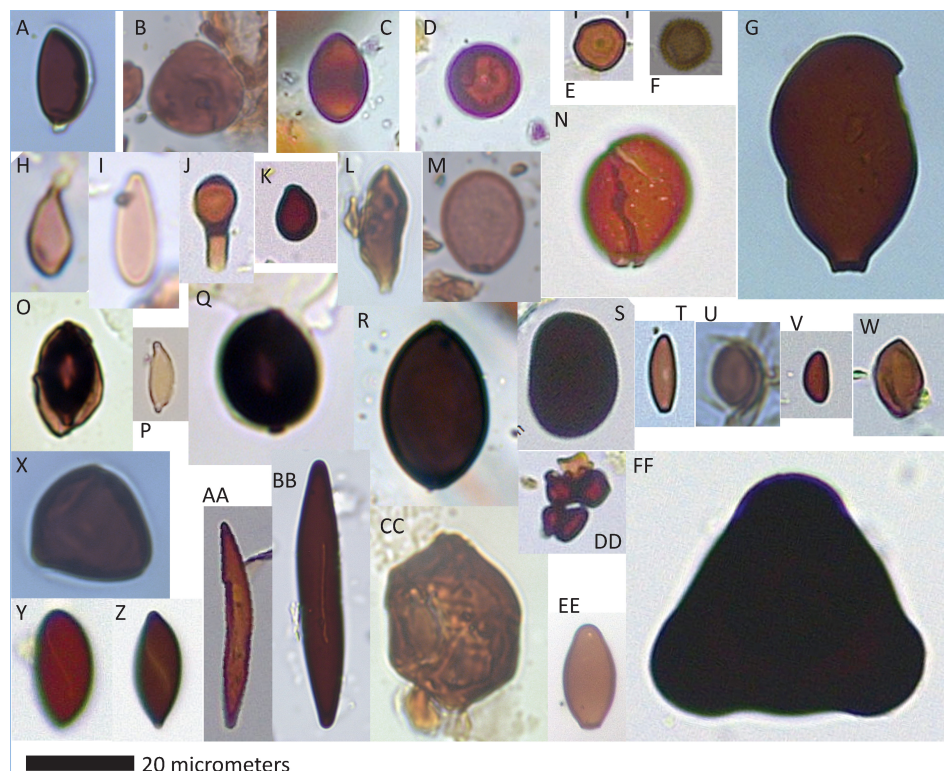


FIGURE 3

Single-celled fungal remains (amero- types). (A) cf. *Acrogenospora*, (B) cf. *Acrogenospora*, (C) BN-2, (D) BN-1, (E) cf. *Gilmaniella*, (F) cf. *Tulostoma*, (G) BN-9, (H) BN-3, (I) BN-35, (J) BN-25, (K) BN-5, (L) *Juglanconis* sp. 1, (M) cf. *Periconia*, (N) *Juglanconis* sp. 2, (O) BN-6, (P) BN-4, (Q) BN-34, (R) cf. *Podospira*, (S) cf. *Nigrospora*, (T) *Annulohyphoxylon* sp., (U) BN-8, (V) *Xylariaceae*, (W) *Chaetomiaceae*, (X) *Catenularia* *elsikii*, (Y) *Helicogermis* sp., (Z) *Rosellinia* aff. *franciscanae*, (AA) cf. *Rosellinia* aff. *necatrix*, (BB) *Kretzschmaria* aff. *deusta*, (CC) BN-11, (DD) BN-37, (EE) cf. *Cercophora* sp., and (FF) *Zopfiella* *neogenica*.

(Beck et al., 2018). This system uses threshold values of modern climatology to define terrestrial areas into five broad classes (tropical, arid, temperate, continental, and tundra), which are then subdivided into 30 sub-types based on seasonal changes in temperature and precipitation (Beck et al., 2018). Fungal palynomorphs identified to extant genus or below were used in the analysis; those identified to family, fossil taxon, or unnamed (assigned a BN- number) were excluded from the analysis. Published occurrences of the NLR of the identified fossil fungi were then compared to the present-day Köppen–Geiger system maps of Beck et al. (2018). All classes where that taxon has been recorded as occurring were then recorded against that fossil fungus providing a 660-point occurrence dataset (Pound et al., 2022). Köppen–Geiger class occurrences for each fossil fungus were then summed for each sample and the class within which all fossil fungi could have co-existed is presented as the reconstruction. Where multiple classes are reconstructed as being possible, all are presented as equally likely. To test how robust the new fungal-based Köppen–Geiger class reconstructions are, we compared modern climatological data from WorldClim2.1 (Fick and Hijmans, 2017) to the present-day distribution of Köppen–Geiger climate

classes and the quantitative palaeoclimate reconstructions of the Kenslow Member that have used three different nearest living techniques (Gibson et al., 2022; McCoy et al., in review).

Results

Fungal assemblages

To date, 110 unique fossil fungal morphotypes (Figures 3–8 and Table 1) have been identified in the Kenslow Member as exposed in Bees Nest pit. Of these, 72 are assigned to extant or known fossil taxa, while an additional 38 have yet to be identified. The assemblages are co-dominated by amero-spores ($n = 36$; Figure 3) and phragmo-spores ($n = 36$; Figures 5, 6). Seven taxa represent epiphyllous structures (Figure 8), while all but one of the remainder are reproductive propagules. That one, *Balaniopsis* sp., is represented by a conidiogenous cell (Figure 7).

The lowermost 2 cm of the Kenslow Member (sample 906) contains a depauperate palynological assemblage

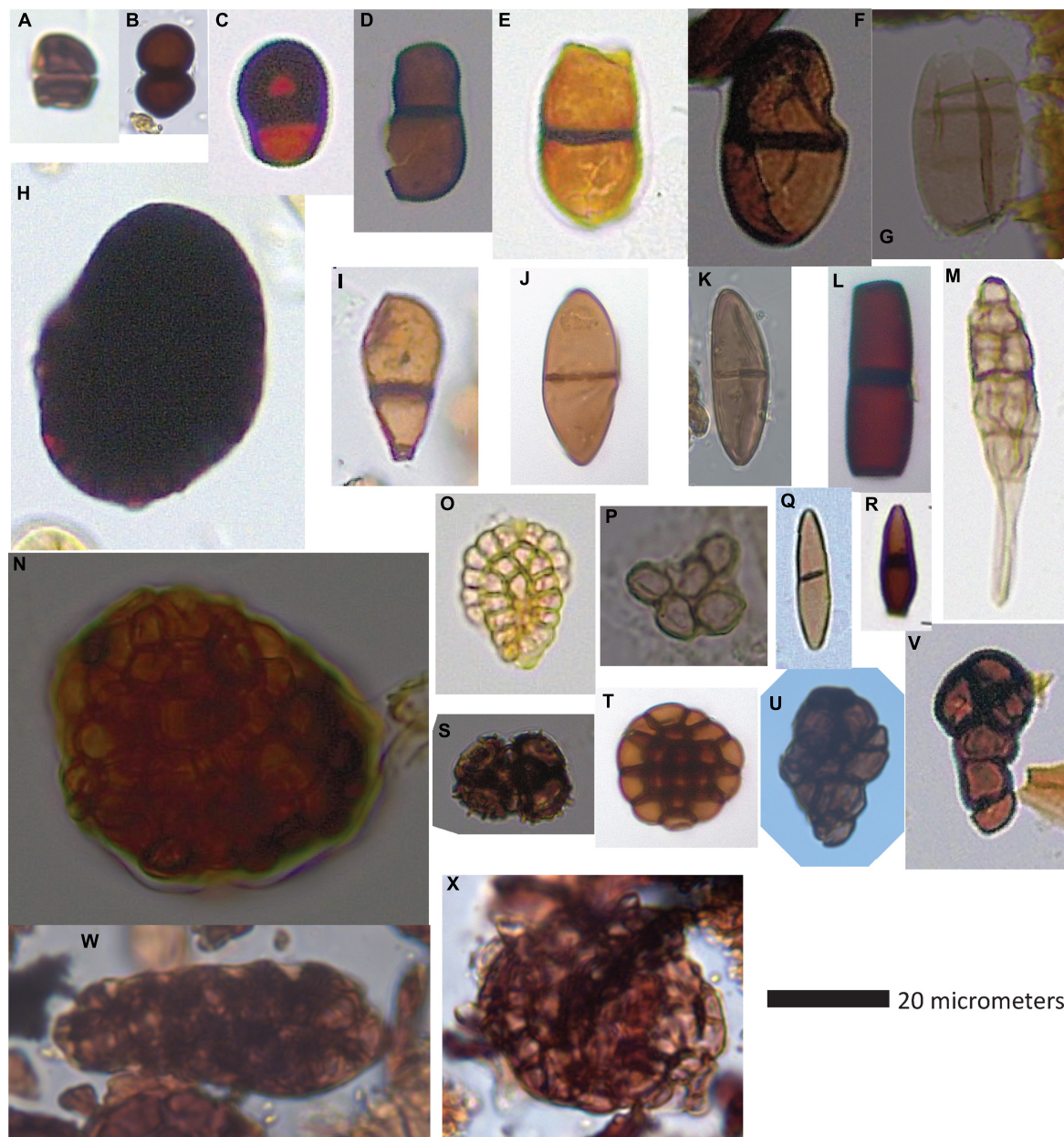


FIGURE 4

Fungal remains with two or more cells (didymo-, dictyo-, broken phragmo-, stauro-, helico-, and bubil- types). (A) BN-7, (B) BN-32, (C) cf. *Endophragmiella*, (D) BN-13, (E) cf. *Ascotaiwania*, (F) BN-36, (G) BN-33, (H) cf. *Ascotaiwania*, (I) BN-38, (J) cf. *Subersisphaeria*, (K) *Delitschia* sp., (L) cf. *Sporoschisma uniseptatum*, (M) *Alternaria* sp., (N) *Endoconidioma* sp., (O) *Dictyosporium* sp., (P) BN-27, (Q) *Amphisphaeria* aff. *millepunctata*, (R) BN-12, (S) *Isthmosphora* aff. *spinosa*, (T) *Acrodictys* sp., (U) *Stemphylium* sp., (V) cf. *Circinoconis*, (W) cf. *Xenosporium*, and (X) BN-26.

that is dominated by charcoal and burnt (i.e., black) pollen. Within this, only fossil cf. *Tulostoma* and *Zopfella neogenica* were identified. Up section, the variety of Non-Pollen Palynomorphs, especially fungi, in the samples increased significantly beginning with sample MPA 68116. Recovery is highly variable through the lower part of the unit (through sample MPA 68112), and

fungi recovered tend to be cosmopolitan saprotrophs, including, *Acrodictys* sp., *Alternaria* sp., and cf. *Tulostoma*.

Sample MPA 68116 contains a higher diversity assemblage, including *Ascobolus* sp., *Rhexoampullifera stogiana*, and several indeterminate taxa (Table 1), while sample MPA 68113 contained an exceptionally depauperate assemblage of only one taxon. Approximately midway up section, beginning with

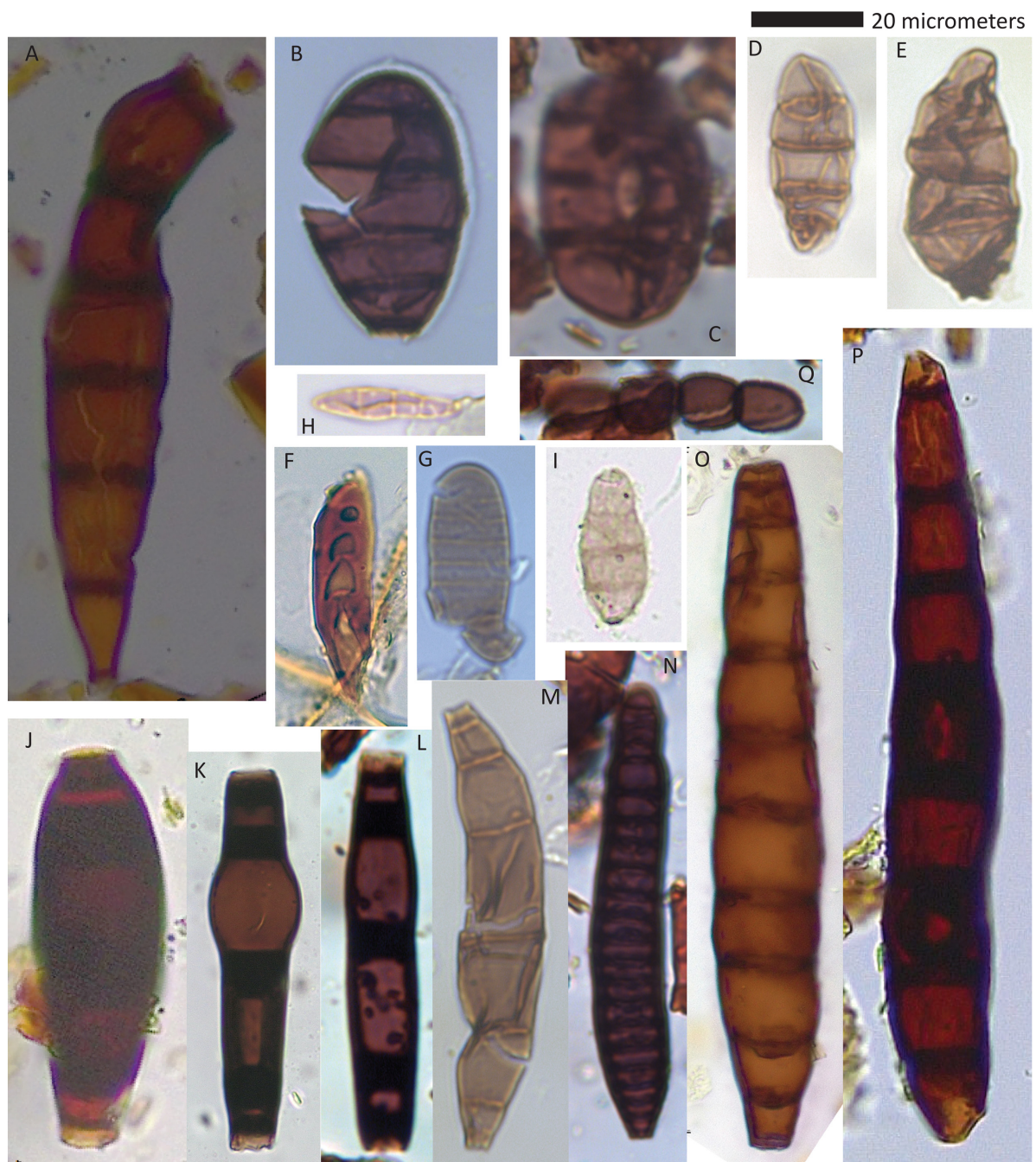


FIGURE 5

Large elongate fungal remains with two or more cells (phragmo- types). (A) cf. *Acrogenotheca*, (B) BN-21, (C) BN-19, (D) cf. *Lophiostoma*, (E) cf. *Pithomyces*, (F) *Dreschlera/Bipolaris*, (G) BN-20, (H) BN-17, (I) BN-23, (J) BN-24 (cf. *Rhexoampullifera*), (K) *Rhexoampullifera sufflata*, (L) *Rhexoampullifera stogieana*, (M) *Sporidesmium* sp. 2, (N) *Ellisembia* sp., (O) cf. *Xylomyces*, (P) *Lobatopedis* sp., and (Q) *Preussia/Sporormiella*.

sample MPA 68111, diversity increased again. Ordination through cluster analysis showed a grouping that can be interpreted biostratigraphically, even though no stratigraphy was provided to the analysis (Figure 9; Supplementary Figure 1). Samples 906 – MPA68112 were grouped and SIMPROF shows this group to be homologous; MPA68113 is the

outgroup due to poor recovery in this sample. All samples above MPA68112 in the sequence were also grouped into a SIMPROF homologous group (Figure 9; Supplementary Figure 1).

Clay from the so-called wood horizon (Pound et al., 2019; O'Keefe et al., 2020) in the middle of the Kenslow

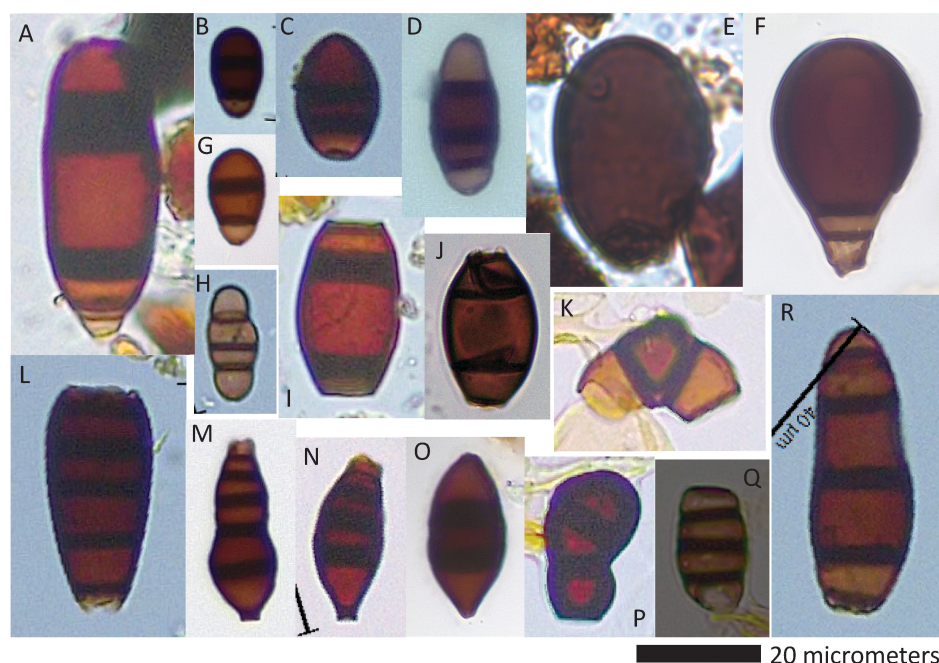


FIGURE 6

Small and medium elongate fungal remains with two or more cells (phragmo-types). (A) cf. *Bactrodesmium* aff. *abruptum*, (B) cf. *Bactrodesmium* sp. 1, (C) cf. *Bactrodesmium* sp. 2, (D) cf. *Bactrodesmium* sp. 3, (E) *Brachysporiella* sp., (F) *Brachysporiella* aff. *setosa*, (G) *Spadicoides* aff. *klotzchii*, (H) cf. *Melanomma*, (I), cf. *Endophragmiopsis* sp. 2, (J) cf. *Endophragmiopsis* sp. 1, (K) *Curvularia* sp., (L) BN-15, (M) *Sporidesmium* aff. *fluminicola*, (N) *Sporidesmium* sp., (O) BN-14, (P) BN-16, (Q) BN-22, and (R) BN-18.

Member (beginning with the Wood Sample) contains abundant fungi, of which 24 were identifiable to modern taxa. These include a number of wet-tolerant taxa, such as cf. *Bactrodesmium* sp. 2, *Diplocladiella* aff. *scabroides*, *Rhexoampulifera* species, cf. *Submersisphaeria* sp., and cf. *Tripodsporium*. Sample 907, obtained from the clay adjacent to wood samples 908–910, contains an interesting assemblage of 9 identifiable taxa, including conidiogenous cells of cf. *Balaniopsis* sp., *Diplocladiella* aff. *scabroides*, indeterminate stalked microthyraceous fungi (BN-28), *Rhexoampulifera stogieana*, cf. *Submersisphaeria*, and *Tetraploa* aff. *artista*, as well as the very enigmatic fungus *Pesavis tagluensis*. Clay from cracks in wood (samples 908–910) recovered from the wood horizon contains a similarly diverse assemblage: abundant members of *Rhexoampullifera*, mostly *R. stogiana*, but also *R. sufflata* and an as-yet un-named form, designated BN-24, and *Tetraploa* aff. *artista* as well as epiphyllous fungi, helicosporous conidia, *Preussia/Sporormiella*, cf. *Submersisphaeria*, and *Zopfella neogenica*, among others (Table 1), although the overall number of named taxa in individual samples is relatively low ($n = 3\text{--}4$, Figure 9).

A metre above the wood horizon (sample 904) and somewhat below that (MPA 63613), fungal diversity again increases, with 25 and 28 identified taxa, respectively (Table 1 and Figure 9). *Rhexoampullifera* and cf. *Submersisphaeria* are still common, although not as common as in the wood

horizon, cf. *Bactrodesmium* become increasingly common. Microthyraceous fungi, including germlings of at least two morphologies, and *Trichothyrium* sp. are very common, as are *Diplocladiella* aff. *scabroides* and other staurospores. The nMDS ordinations showed a grouping of samples processed from sediment associated with fossil wood samples (Supplementary Figure 2). However, the ANOSIM test showed that neither sample type (Global $R = 0.062$, Significance 29.2%), or processing method – see Supplementary Table 1 (Global $R = -0.025$, Significance 55%) controlled the similarity of fungal palynomorph assemblages.

Climate reconstruction

The new fungal-based reconstructions show the presence of a Cfb (temperate, no dry season, warm summer) climate during Kenslow Member deposition (Figure 9). For the number of fungal taxa used in the reconstruction, the reader is referred to Figure 9. This class is reconstructed for all assemblages. In the upper part of the section, four of the ten assemblages reconstruct the Cfb class as the only possible class within which all the modern relatives of the fossil fungi could co-occur (Figure 9). Where multiple classes are equally possible (all taxa reconstruct multiple classes) for the other assemblages, these are the Cfb, Cfa (temperate, no dry season, hot summer) and

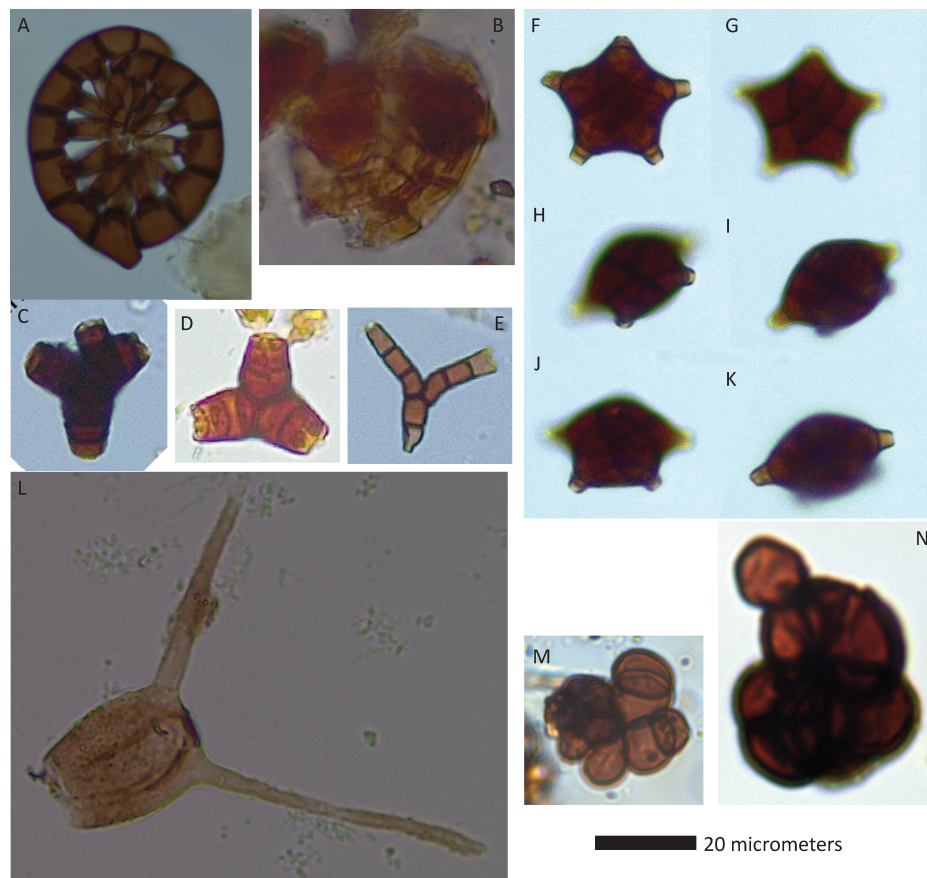


FIGURE 7

Coiled, star-shaped, and otherwise complex fungal remains (helico-, stauro-, and other types). (A) *Pesavis tagluensis*, (B) cf. *Ctenosporium*, (C) *Tetrabrunneospora* aff. *ellisii*, (D) cf. *Triposporium*, (E) *Diplocradiella* aff. *scalaroides*, (F–K) successive views of a single conidiogenous cell of cf. *Balaniopsis* mounted in glycerine, (L) *Tetraploa aristata*, (M) BN-30, (N) BN-29.

the Aw (Tropical, savannah). In the lower part of the section, as defined by the cluster analysis, all assemblages reconstruct multiple Köppen–Geiger classes, but always contain the Cfb class (Figure 9).

Discussion

Fungi-based palaeoenvironmental reconstruction

The main cluster split noted in Figure 9 and Supplementary Figure 1 appears to roughly correspond to the CONISS-indicated boundary between KM19-1 (lower portion of the section – a low diversity conifer dominated pollen assemblage) and KM19-2 (upper portion of the section – a more diverse mixed forest assemblage) pollen zones of McCoy *et al.* (in review). This boundary separates more (zone KM19-2) and less (zone KM19-1) diverse plant pollen and spore assemblages that appear to correspond to initiation of

what would become a peat-producing wetland system. The change is coupled with increased coalification of preserved wood (O’Keefe *et al.*, 2020) and an increase in freshwater submerged fungal taxa (cf. *Bactrodesmium*, *Brachysporiella* aff. *setosa*, *Rhexoampullifera* species, and stauro-forms, including *Diplocradiella* aff. *Scalaroides*).

Palaeoclimate reconstruction from fossil fungi

The climate reconstruction of Cfb places the Miocene UK in the same Köppen–Geiger climate classification as present-day (Beck *et al.*, 2018). The Cfb class is defined by a hottest month temperature of < 22°C (Peel *et al.*, 2007; Beck *et al.*, 2018). Plant-based co-existence approach reconstructions from the Kenslow Member at Bees Nest Pit of warmest month temperature produce temperatures warmer than this at 26.5–28.3°C (Pound and Riding, 2016), 21.6–28.1°C (Gibson *et al.*, 2022), and 24.7–27.9°C (McCoy *et al.*,

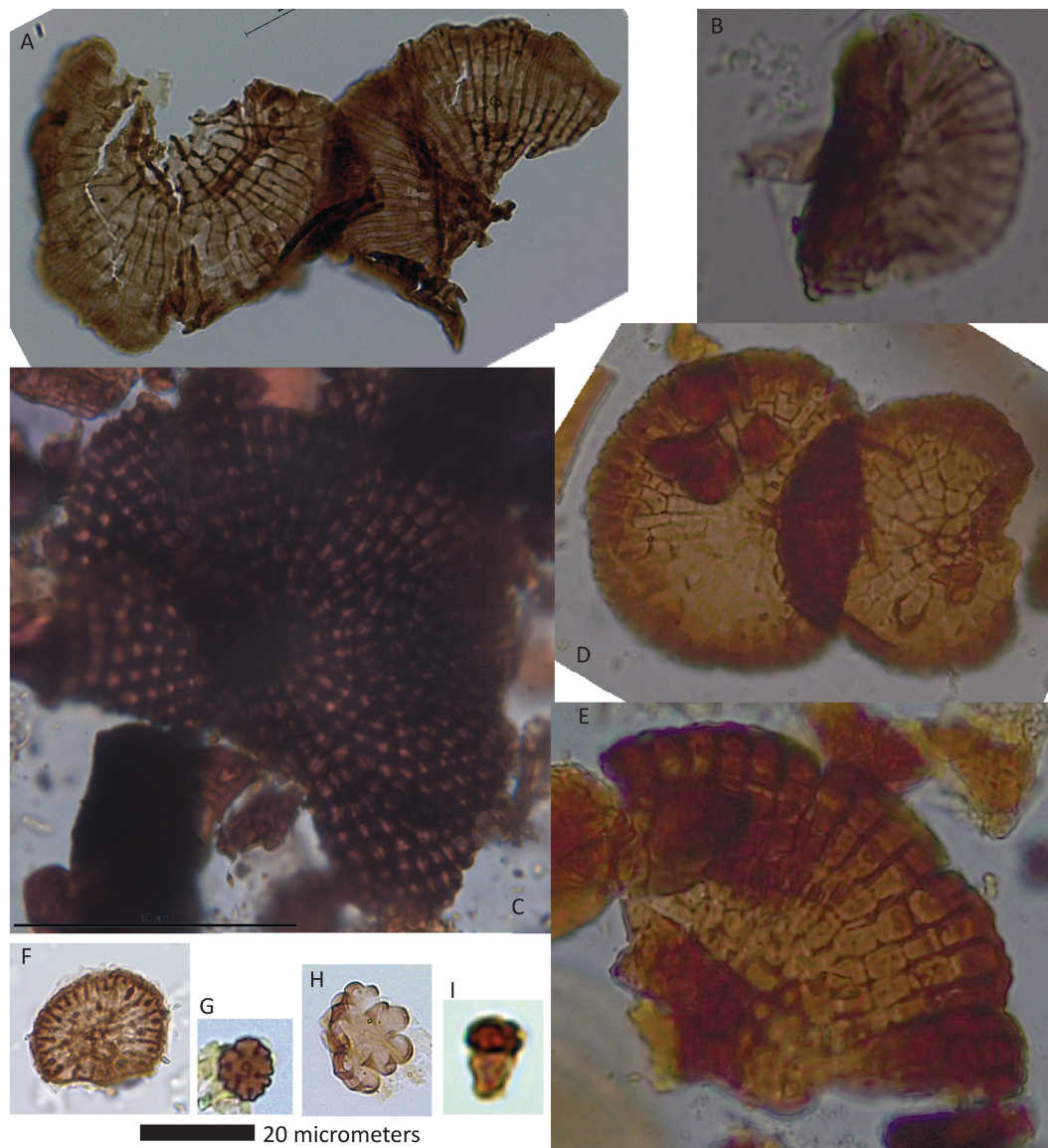


FIGURE 8

Epiphyllous fungi. (A) *Neomycoleptodiscus* sp., (B) BN-28, (C) *Trichothyrium* sp., (D) *Microthyrium* sp. 2, (E) *Microthyrium* sp. 1, (F) microthyraceous germling, (G) microthyraceous germling sp. 2, (H) microthyraceous germling sp. 1, and (I) BN-31.

in review). However, statistical techniques produce warmest quarter temperatures in line with anticipated values for a Cfb class – $21.9^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ and $20.6^{\circ}\text{C} \pm 5.8^{\circ}\text{C}$ (Gibson et al., 2022). That said, by definition, the warmest quarter should be colder than the hottest month. Comparison of the palaeobotanical palaeoclimate reconstructions to the present-day climatology of the Cfb, and warmer Cfa, Köppen–Geiger climate classes shows that the Bees Nest Pit reconstructions are positioned at the upper limits of the Cfb class and lower edge of the Cfa class (Figure 10). The co-existence approach estimate lies within the Cfa Köppen–Geiger climate class (Figure 10), but is known to be estimating warmer than actual

temperatures due to the underlying dataset (Gibson et al., 2022). The present-day United Kingdom climate for Derbyshire (based on WorldClim2.1) is positioned toward the cooler end of the Cfb Köppen–Geiger climate class (Figure 10). All previous palaeoclimate reconstructions also show that winter temperatures were above 0°C , helping to support a classification in the Cfb class (Pound and Riding, 2016; Gibson et al., 2022; McCoy et al., *in review*).

The fungi-based reconstruction of a Cfb Köppen–Geiger climate classification also implies minimal differences in precipitation seasonality (Beck et al., 2018). Using co-existence approach reconstructions, Pound and Riding (2016)

TABLE 1 Taxonomy and occurrence of fungi present in samples from Bees Nest.

Division	Class	Family	Identified taxa	905	906	68116	68115	68113	68112	68111	Wood	907	908	909	910	68110	68109	65716	63613	904
Ascomycota	Sordariomycetes	Acrodictyceae	<i>Acrodictys</i> sp.					1		1									1	1
Ascomycota	Dothideomycetes	Pleosporaceae	<i>Alternaria</i> sp.			1			1										1	
Ascomycota	Sordariomycetes	Amphisphaeriaceae	<i>Amphisphaeria</i> aff. <i>millepunctata</i>																	1
Ascomycota	Sordariomycetes	Hypoxyaceae	<i>Annulohypoxylon</i> sp.																	1
Ascomycota	Pezizomycetes	Ascobolaceae	<i>Ascobolus</i> sp.			1														
Ascomycota	Dothideomycetes		<i>Bactrodesmium</i> aff. <i>abruptum</i>																1	
Ascomycota	Sordariomycetes		<i>Brachysporiella</i> aff. <i>setosa</i>												1				1	1
Ascomycota	Sordariomycetes		<i>Brachysporiella</i> sp.								1									
Ascomycota	Sordariomycetes	Chaetosphaeriaceae	<i>Catenularia</i> <i>elsikii</i>								1								1	
Ascomycota	Dothideomycetes	Acrogenosporaceae	cf. <i>Acrogenospora</i> sp. 1							1										
Ascomycota	Dothideomycetes	Acrogenosporaceae	cf. <i>Acrogenospora</i> sp. 2								1									
Ascomycota	Dothideomycetes	Incertae sedis	cf. <i>Acrogenotheca</i>								1									
Ascomycota	Sordariomycetes	Savoryellaceae	cf. <i>Ascotaiwania</i>												1		1			
Ascomycota	Dothideomycetes		cf. <i>Bactrodesmium</i> sp. 1								1	1								
Ascomycota	Dothideomycetes		cf. <i>Bactrodesmium</i> sp. 2								1								1	1
Ascomycota	Dothideomycetes		cf. <i>Bactrodesmium</i> sp. 3							1										1
Ascomycota			cf. <i>Balaniosis</i>									1								
Ascomycota	Sordariomycetes	Lasiosphaeriaceae	cf. <i>Cercophora</i>																	1
Unknown			cf. <i>Circinoconis</i>																1	
Ascomycota			cf. <i>Ctenosporium</i>								1									
Ascomycota	Dothideomycetes	Dictyosporiaceae	cf. <i>Dictyosporium</i>														1			
Ascomycota	Dothideomycetes	Dothideaceae	cf. <i>Endoconidioma</i>														1			
Ascomycota	Sordariomycetes	Helminthosphaeriaceae	cf. <i>Endophragmiella</i>														1			
Ascomycota			cf. <i>Endophragmiopsis</i> sp. 1															1	1	
Ascomycota			cf. <i>Endophragmiopsis</i> sp. 2																1	
Ascomycota			cf. <i>Gilmaniella</i>													1	1		1	1
Ascomycota	Dothideomycetes	Lophiostomataceae	cf. <i>Lophiostoma</i>																1	
Ascomycota	Dothideomycetes	Melanommataceae	cf. <i>Melanomma</i>																	1
Ascomycota	Sordariomycetes	Apiosporaceae	cf. <i>Nigrospora</i>							1		1							1	1
Ascomycota	Dothideomycetes	Periconiaceae	cf. <i>Periconia</i>								1									
Ascomycota	Dothideomycetes	Astrosphaeriellaceae	cf. <i>Pithomyces</i>														1			
Ascomycota	Sordariomycetes	Podosporaceae	cf. <i>Podospora</i>								1									
Ascomycota	Sordariomycetes	Xylariaceae	cf. <i>Rosellinia</i>																	1
Ascomycota	Sordariomycetes	Annulatascaceae	cf. <i>Submersisphaeria</i>								1	1		1						1
Ascomycota	Leotiomycetes	Asterinaceae	cf. <i>Triposporium</i>								1									
Basidiomycota	Agaricomycetes	Agaricaceae	cf. <i>Tulostoma</i>		1	1	1		1										1	

(Continued)

TABLE 1 Continued

Division	Class	Family	Identified taxa	905	906	68116	68115	68113	68112	68111	Wood	907	908	909	910	68110	68109	65716	63613	904
Ascomycota	Dothideomycetes	Tubeufiaceae	cf. <i>Xenosporium</i>							1								1	1	
Ascomycota	Dothideomycetes	Aliquandostipitaceae	cf. <i>Xylomyces</i>						1											1
Ascomycota	Pezizomycetes	Ascobolaceae	Chaetomiaceae									1							1	1
Ascomycota	Lecanoromycetes	Micropeltidaceae	<i>Chaetothyria</i> sp.							1										
Ascomycota	Dothideomycetes	Pleosporaceae	<i>Curvularia</i> sp.							1							1			
Ascomycota	Dothideomycetes	Sporormiaceae	<i>Delitschia</i> sp.											1					1	
Ascomycota			<i>Diplocladiella</i> aff. <i>scalaroides</i>								1	1								1
Ascomycota	Dothideomycetes	Pleosporaceae	<i>Drechslera/Bipolaris</i>																1	
Ascomycota	Sordariomycetes		<i>Ellisembia</i> sp.														1		1	
Ascomycota	Sordariomycetes	Xylariaceae	<i>Helicogermis</i> sp.																	1
Ascomycota	Dothideomycetes	Microthyriaceae	<i>Isthmospora</i> aff. <i>spinosa</i>																1	
Ascomycota	Sordariomycetes	Juglanconidaceae	<i>Juglanconis</i> sp. 1																1	
Ascomycota	Sordariomycetes	Juglanconidaceae	<i>Juglanconis</i> sp. 2								1									
Ascomycota	Sordariomycetes	Xylariaceae	<i>Kretzschmaria</i> aff. <i>deusta</i>								1		1							
Ascomycota			<i>Lobatopedis</i> sp.												1					
Ascomycota	Dothideomycetes	Microthyriaceae	Microthyraceous germings										1						1	1
Ascomycota	Dothideomycetes	Microthyriaceae	<i>Microthyrium</i> sp. 1								1									
Ascomycota	Dothideomycetes	Microthyriaceae	<i>Microthyrium</i> sp. 2								1									
Ascomycota	Dothideomycetes	Muyocoproneae	<i>Neomycocleptodiscus</i> sp.														1		1	
Ascomycota	Dothideomycetes	Sporormiaceae	<i>Pesavis tagluensis</i>									1								
Ascomycota			<i>Preussia/Sporormiella</i>																1	1
Ascomycota			<i>Rhexoampullifera stogiana</i>			1					1	1	1	1	1		1		1	1
Ascomycota			<i>Rhexoampullifera sufflata</i>								1	1				1			1	
Ascomycota	Sordariomycetes	Xylariaceae	<i>Rosellinia</i> aff. <i>francisciae</i>																	1
Ascomycota	Sordariomycetes	Xylariaceae	<i>Rosellinia</i> aff. <i>necatrix</i>																	1
Ascomycota	Sordariomycetes	Xenospadicoidaceae	<i>Spadicoides</i> aff. <i>klotzschii</i>								1									1
Ascomycota	Sordariomycetes	Sporidesmiaceae	<i>Sporidesmium</i> aff. <i>flumincola</i>								1									1
Ascomycota	Sordariomycetes	Sporidesmiaceae	<i>Sporidesmium</i> sp. 1																	1
Ascomycota	Sordariomycetes	Sporidesmiaceae	<i>Sporidesmium</i> sp. 2																1	1
Ascomycota	Sordariomycetes	Chaetosphaeriaceae	<i>Sporoschisma</i> aff. <i>uniseptatum</i>								1									1
Ascomycota	Dothideomycetes	Pleosporaceae	<i>Stemphylium</i> sp.															1		
Ascomycota			<i>Tetrabruneospora</i> aff. <i>ellisii</i>																	1
Ascomycota	Dothideomycetes	Tetraplosphaeriaceae	<i>Tetraploa aristata</i>				1				1									
Ascomycota	Dothideomycetes	Trichothyriaceae	<i>Trichothyrium</i> sp.							1		1	1	1		1	1	1	1	1
Ascomycota	Sordariomycetes	Xylariaceae	Xylariaceae											1				1	1	1

(Continued)

TABLE 1 Continued

Division	Class	Family	Identified taxa	905	906	68116	68115	68113	68112	68111	Wood	907	908	909	910	68110	68109	65716	63613	904
Ascomycota	Sordariomycetes	Lasiosphaeriaceae	Zopfiella neogenica		1															
			BN-1						1								1	1	1	
			BN-2								1									
			BN-3								1									
			BN-4								1									
			BN-5																1	1
			BN-6						1											
			BN-7													1				
			BN-8																1	
			BN-9										1							
			BN-10																	
			BN-11															1	1	
			BN-12									1								
			BN-13														1			
			BN-14																	1
			BN-15																1	1
			BN-16														1			
			BN-17						1							1				
			BN-18																	1
			BN-19								1									
			BN-20																1	
			BN-21							1										
			BN-22														1			
			BN-23			1														
			BN-24								1									
			BN-25																	
			BN-26								1									
			BN-27			1														
			BN-28				1													
			BN-29															1		
			BN-30								1									
			BN-31															1		1
			BN-32										1							
			BN-33			1														
			BN-34				1													
			BN-35								1									
			BN-36																1	
			BN-37																	1
			BN-38								1									

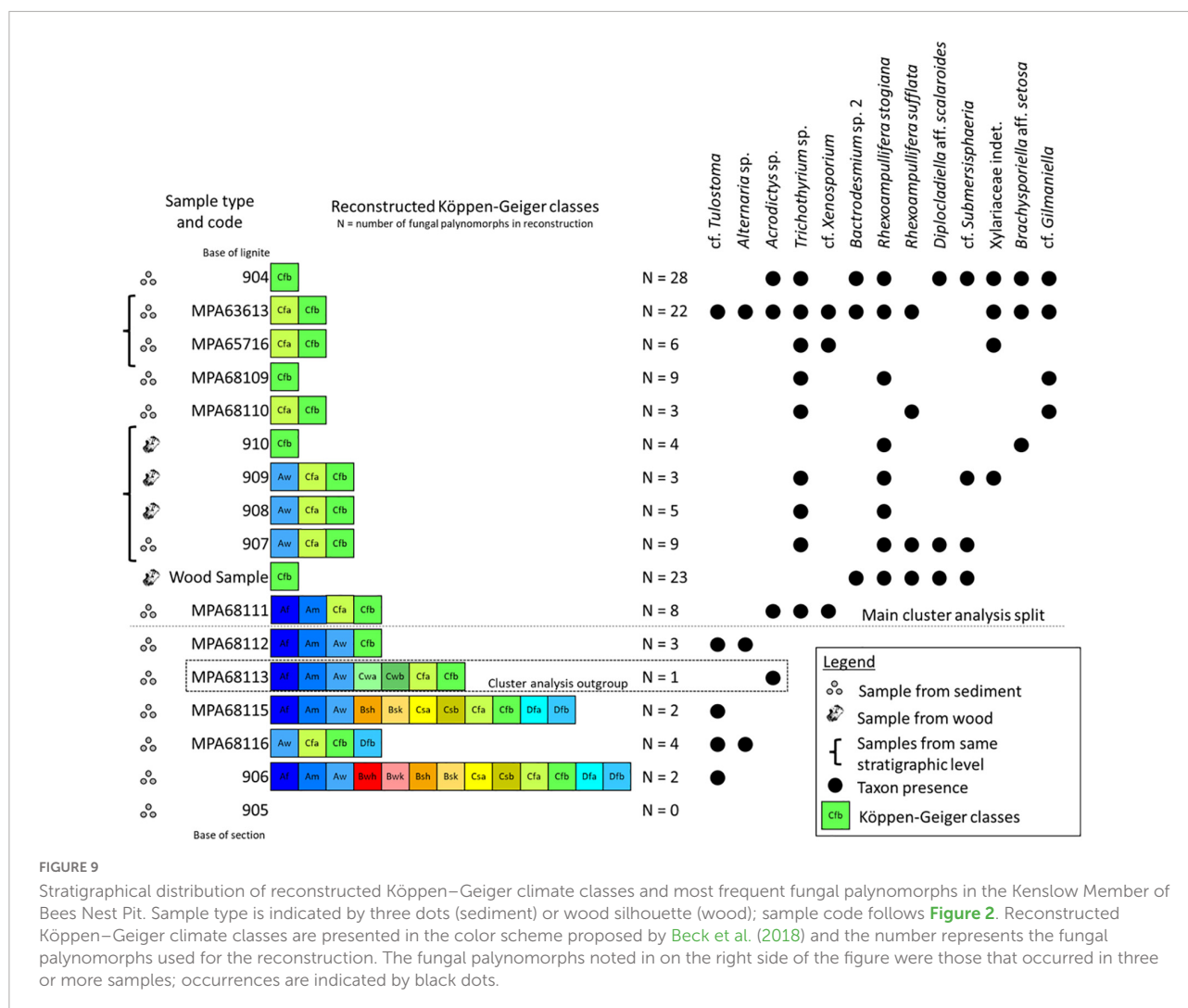


FIGURE 9

Stratigraphical distribution of reconstructed Köppen–Geiger climate classes and most frequent fungal palynomorphs in the Kenslow Member of Bees Nest Pit. Sample type is indicated by three dots (sediment) or wood silhouette (wood); sample code follows Figure 2. Reconstructed Köppen–Geiger climate classes are presented in the color scheme proposed by Beck et al. (2018) and the number represents the fungal palynomorphs used for the reconstruction. The fungal palynomorphs noted in on the right side of the figure were those that occurred in three or more samples; occurrences are indicated by black dots.

had proposed a degree of seasonality for the Kenslow Member at Bees Nest Pit. However, reconstructions using statistical techniques show relatively low seasonality with high MAP (Gibson et al., 2022). Co-existence approach reconstructions agree with relatively high MAP, with all techniques showing it was likely greater than 1000 mm per year (Pound and Riding, 2016; Gibson et al., 2022; McCoy et al., in review). The seasonality values presented by Gibson et al. (2022) all fall within the values experienced by the present-day Cfb Köppen–Geiger climate class (Fick and Hijmans, 2017; Beck et al., 2018). The consistency between the fungi-based reconstruction and multiple palaeobotanical techniques shows the applicability of fossil fungi to reconstructing qualitative palaeoclimate information.

A Cfb Köppen–Geiger climate classification for the late Middle Miocene of Derbyshire is consistent with reconstructions from plant fossils on Iceland for the Middle to Late Miocene as well (Denk et al., 2013). This has been

proposed as showing the presence of the Gulf Stream, which would have also contributed to the warmth and humidity of the United Kingdom during the deposition of the Kenslow Member. Sea surface temperature data points to a flat latitudinal temperature gradient in the North Atlantic during this time interval, that might also indicate a proto-North Atlantic Current (Super et al., 2020). Reconstructed temperatures from the Netherlands, based on branched glycerol dialkyl glycerol tetraethers also show, probably, warm month temperatures of 21.5–25°C (Donders et al., 2009; Wang et al., 2018; Crampton-Flood et al., 2020).

Broader implications and future work

The new fungal assemblage from the Kenslow Member contains two genera with multiple species that facilitates dating constraints on the phylogenetic studies of these. In the phylogeny of Voglmayr et al. (2019) the presence of two

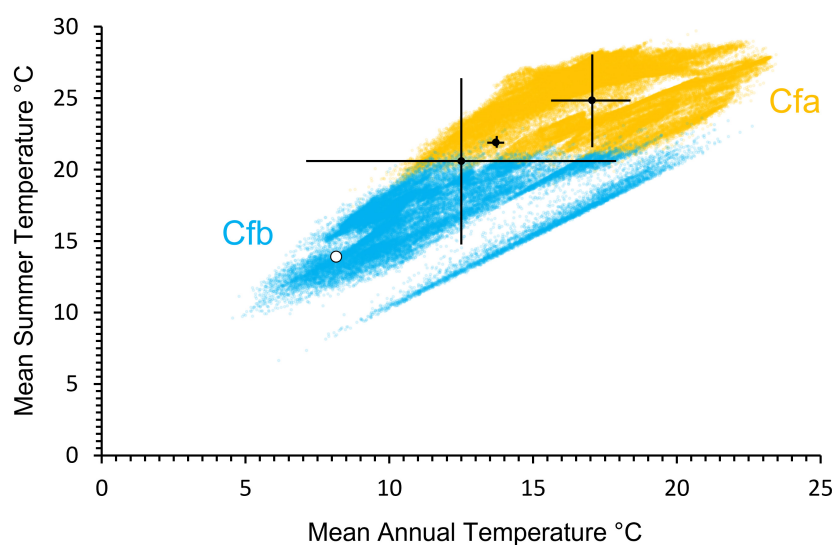


FIGURE 10

Climatological distribution of the present-day Cfa (yellow) and Cfb (blue) Köppen–Geiger climate classes based upon mean annual temperature (x-axis) and summer temperature (y-axis). The white dot shows the present-day values for Derbyshire, United Kingdom. The black dots with error bars are paleobotanical based reconstructions from Gibson et al. (2022) and McCoy et al. (in review). The present-day dataset comprises 39,646 data points using Köppen–Geiger climate classifications from Beck et al. (2018) and WorldClim2.1 data from Fick and Hijmans (2017).

species of *Juglanconis* identified from the Kenslow Member of Bees Nest Pit shows *Juglanconis* had evolved by this point. There was significant diversification of the *Juglans* clade during the Miocene (Mu et al., 2020) and *Juglans* pollen is reported from the same section at Bees Nest Pit (McCoy et al., in review). The presence of both the fungus and the host could be showing co-evolution over the course of the Cenozoic and would be worthwhile investigating fossil *Juglanconis* co-occurrence with *Juglans* during earlier time intervals. Species of *Rosellinia* diverged from those of *Kretzschmaria* during the Paleogene at around 50 million years ago (Samarakoon et al., 2016). The presence of *Rosellinia* aff. *francisciae* and *R. aff. necatrix* in the 12 Ma Kenslow Member of Bees Nest Pit shows these had originated by this time (Table 1). In the undated phylogeny of Bahl et al. (2005) these two species are on distinct branches, which suggests that this radiation had occurred earlier than the late Middle Miocene.

The presence of *Pesavis tagluensis* in sediments from the Bees Nest Pit extends the stratigraphic range of this taxon significantly. *Pesavis tagluensis* is an accepted biostratigraphical indicator for the Maastrichtian to Paleogene (Kalgutkar and Sweet, 1988). This fungal remain has a range of known sizes, with smaller forms (averaging 32 μm) known from the Maastrichtian through middle Paleocene, and larger forms more frequently found in the late Paleocene to Eocene. The species of *P. tagluensis* from the Kenslow Member is 43.5 μm tall \times 38.3 μm wide, placing it in the middle of the range (21–60 μm), congruent with middle-sized Paleocene

and Eocene specimens (Kalgutkar and Sweet, 1988). In the United Kingdom, *Pesavis* has been found previously in the Ardtun Leaf Beds on the Isle of Mull (Selandian), the Reading Beds in Berkshire (Thanetian-earliest Ypresian) and Hordle Cliff in Hampshire (Priabonian) (Smith, 1978; Smith and Crane, 1979; King, 2016; Arting et al., 2018). No other taxon present in the Kenslow Member is suggestive of a Paleogene age; indeed, paleobotanical and plant palynological remains clearly suggest that it is Serravallian (Pound and Ridding, 2016), therefore, the range of *Pesavis* is greater than previously known, extending to at least 12 Ma. Frederiksen et al. (1996) noted that *Pesavis* in Alaska is associated with wood-rich horizons; it likewise occurs in a wood-rich zone in the Kenslow Member. The Oligocene – Miocene palynology of the United Kingdom is underexplored; it is strongly suggested that fungal palynology be completed as part of all future studies to refine the distribution and ecology of this intriguing group.

Conclusion

A total of 110 unique fossil fungal palynomorph morphotypes were recorded from the Kenslow Member as exposed in Bees Nest Pit, Brassington, Derbyshire, United Kingdom. Samples were co-dominated by amero spores and phragmo spores, of which 72 (roughly 2/3) have been identified to extant taxa (Table 1). The majority of taxa encountered occur in association with wood-rich horizons.

Neither sediment type nor processing method (mineral-acid vs. acid free) had an appreciable impact on fungal recovery.

Fossil fungal assemblages work for a palaeoclimate proxy. The assemblages throughout the Kenslow Member as exposed at Bees Nest Pit are indicative of deposition in Köppen–Geiger class Cfb – temperate, no dry season, warm summer. The results compare well to previously published quantitative paleobotanical and palynological climate reconstruction for the well-studied Kenslow Member, and together they are suggestive of the upper end of the Cfb climate range. Of note, it is clear from this study that the Serravallian of the United Kingdom was unlikely to be seasonally dry.

This study represents a first step in the utilization of fossil fungal remains as palaeoclimate proxies in the Neogene and lays the foundation for continued work in this area.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.5281/zenodo.6558518>.

Author contributions

MP and JO’K conceived the idea for and designed the study. MP, JO’K, ML, and JR completed the fieldwork and sample collection for the study. JR and MP provided the background information for the study. JO’K and MP generated the data for the study. NN, JO’K, IR, and MP completed the taxonomic identifications. MP developed the NLR technique and determined ecological tolerances and climatological zonations. MP and JO’K completed the data analysis with support of IR. MP and JO’K drafted the figures. MP and JO’K equally led the initial writing with support of NN and contributions from IR and JR. MP, JO’K, and IR led the manuscript editing with supporting editing by all other authors. All authors contributed to the article and approved the submitted version.

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World (FiaWW)” (NSF award #2015813 to JO’K and NERC award identifier NE/V01501X/1 to MP).

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2022.947623/full#supplementary-material>

SUPPLEMENTARY TABLE 1
Sample information.

SUPPLEMENTARY TABLE 2
Descriptions of BN taxa.

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