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A comparative study of optical properties of NaOH peat extracts: implications for humification studies

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Abstract: Assessment of the degree of decay of peat (humification) in ombrotrophic mires has become a standard technique for palaeoclimatic reconstruction, based on the finding that decay is primarily determined by surface wetness and temperature at the time of peat deposition. Determination of humification is undertaken by colorimetric measurement of an alkali extract of the peat at 540 nm. Humification is proportional to the amount of humic matter dissolved by this extraction process, although few researchers convert results to a quantitative measure of humification expressing results as percentage light transmission through the peat. This paper uses luminescence spectroscopy to assess the chemical composition of these extracts. Luminescence excitation and emission wavelengths suggest that high molecular weight acids ('humic acids') are altered by the extraction procedure to form lower molecular weight acids ('fulvic acids'), amino acids and polysaccharides. Percentage transmission is principally related to luminescence emission wavelength and thus to molecular weight of the compounds present. Luminescence emission shows much more sensitivity to peat composition and demonstrates that different plant species may be affected to different degrees by the NaOH extraction process. The findings broadly support the underlying principle of colorimetric determination of 'humification' whereby transmission levels decrease with increasing plant breakdown, but show that it is based on an inadequate understanding of the chemical processes occurring in peat decay and preparation procedures. Luminescence spectroscopy provides a technique for resolving these issues.

Key words: Peat, humification, luminescence, humic acid, fulvic acid, palaeoclimate, Ireland.

Introduction

Analysis of proxies of peat humification is one of a range of methods utilized in studies of ombrotrophic peat bogs as potential archives of palaeoclimatic and palaeoenvironmental data. Peat humification has been used for several decades (Bahnson, 1968; Casparie, 1972; Aaby and Tauber, 1975; Aaby, 1976; 1986; Blackford and Chambers, 1991; 1993; 1995; Chambers *et al*., 1997; Baker *et al.*, 1999) and has the advantages of being relatively simple and quick, and applicable to blanket as well as raised mire peats (Chambers *et al*., 1997). The basic principle of the technique has been most clearly defined by Blackford and Chambers (1993: 11): 'Humic acids are produced by the decomposition of organic material. They are dark brown in solution, giving humus its colour. As peat decomposes, the proportion of humic acid increases, and attempts have been made to estimate the quantities of humic acid in peat and organic soil. The extractant most widely used . . . has been sodium hydroxide. Studies . . . have assumed that the colour of the NaOH extracts are indicative of the degree of humification, and therefore of the extent of decomposition'. As decomposition is primarily a function of the degree of surface wetness of the bog, and surface wetness on ombrotrophic mires is determined by climate, the humification record represents a palaeoclimatic record, although there may also be some influence of botanical composition of the peat. However, humic acids in peats and the chemical transformations that take place on decomposition are complex and poorly understood (Blackford and Chambers, 1993), although there are now some data for similar processes of soil organic matter (SOM) degradation (Gruggenberger and Zech, 1994a; 1994b; Pohhacker and

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Zech, 1995; Sanger *et al.*, 1997; Zech *et al.*, 1997). Despite uncertainties over the processes involved, and evidence that NaOH and KOH extraction can preferentially release high molecular weight fractions in *Sphagnum* peat but not *Carex* peat (Garcia *et al.*, 1993), colorimetric determination of the degree of peat humification has remained a widely used and extremely valuable technique in palaeoclimatic studies. This paper uses luminescence properties of NaOH extracts from peat to determine the acid structures in more detail and to assess the implications for humification studies in palaeoclimatic research. It presents results from a test profile for which luminescence properties are compared to conventional humification measurements. The principal aim is to examine changes in luminescent properties across peat of contrasting humification characteristics to see whether the underlying principles on which humification studies are based can be supported in terms of the acid structures identifiable through luminescence analysis.

Luminescence spectroscopy

Luminescence occurs when molecules release energy in the form of light, after having been previously excited by a high-energy light source that raised the energy levels of the electrons within the molecule. The emitted luminescent light is at a longer wavelength than the excitation light. Luminescence in the natural environment is predominantly generated by organic acids (humic and fulvic) and amino-acid groups within proteins, which derive from decomposed plant and animal material in soils (Senesi *et al.*, 1991; Burstein and Emelyanenko, 1996). Inorganic minerals may occasionally luminesce (e.g., Mn^{2+} in calcite; Pedone *et al.*, 1990), and recent studies have investigated anthropogenically generated luminescent pollutants (e.g., Kotzick and Niesser, 1996). Many studies have investigated the luminescence of organic matter extracted from soils and dissolved organic matter in surface and groundwaters (Coble *et al.*, 1990; Senesi *et al.*, 1991; De Souza Sierra *et al.*, 1994; Coble, 1996; Mobed *et al.*, 1996; Luster *et al.*, 1996; Erich and Trusty, 1997; Ohno and Cronan, 1997; Baker *et al.*, 1997; 2000; Baker and Barnes, 1998; Baker and Genty, 1999). These studies have revealed the presence of several luminescence intensity peaks at excitation and emission wavelength pairs of 250–260:380–460 nm, 300–340:410–480 nm, 270–280:300–360 nm and 250–260:300–360 nm. The first two have been ascribed to an organic acid (humic or fulvic acid) source, and the last two to amino-acid groupings within proteins. Previous studies have suggested that organic acids from different sources (e.g., marine versus terrestrial; Coble, 1996) as well as from different soil types and of different molecular weight (Miano *et al.*, 1988; Senesi *et al.*, 1991; Barancíková *et al.*, 1997; Rivero *et al.*, 1998) may be differentiated by their luminescence properties. Two distinct groups of organic acids can be identified: (1) high molecular weight organic acids (typically >10 000 daltons molecular weight – 'humic acids' (HA)) with a high excitation and emission wavelength of luminescence; (2) low molecular weight organic acids (<2000 daltons molecular weight, 'fulvic acids' (FA)) with a lower excitation and emission wavelength of luminescence.

These differences in luminescence properties are due to an increase in the degree of aromacity, the content of carboxylic groups and polycondensed aromatic and conjugated structures within the high molecular weight acids (Miano *et al.*, 1988; Senesi *et al.*, 1991). Changes in the development of soil humification are reflected in changes in the composition (molecular weight or degree of aromaticity) of the soil-dissolved organic acids (Zech *et al.*, 1992; 1997; Christ and David, 1996), and thus their luminescence properties.

A particular advantage of the approach used here is that very low concentrations of fluorescent compounds can be detected, much lower than those required for absorption spectrophotometry, and 'the absorption spectrum of one component of a mixture of absorbing compounds can be picked out by tuning to the wavelength of the appropriate fluorescence emission band' (Parker, 1968: 21). Thus, in peat extracts which will have a mix of such signals, this technique can separate out the relative importance of the different absorbing fluorescing compounds (or indeed show their presence/absence), which as a whole produce the humification signal defined by the standard colorimetric technique.

Extracts from peat that is 'poorly humified' would be expected to have a greater proportion of high molecular weight, highly aromatic, organic carbon fraction ('HA'), and therefore a higher wavelength of luminescence excitation and emission than that from 'well humified' peat with a low molecular weight organic acid composition ('FA'). As well as variations in luminescence wavelength, organic acid luminescence intensity can also be determined. In soil extracts and water samples, luminescence intensity of the fulvic and humic acid-like luminescence centres is correlated with Total Organic Carbon (TOC) (Mobed *et al.*, 1996), because 40–60% of all TOC is luminescent (Senesi, 1993). Therefore the analysis of luminescence intensity of peat extracts permits the assessment of the amount of TOC per unit mass in the form of luminescent organic acids, which may be affected by the degree of humification.

Luminescence analysis therefore has the potential to improve our understanding of the precise signal contained in plant humification extracts. Luminescence wavelength variations of these extracts should provide information on the molecular weight and chemical structure of dissolved organic acids, whereas luminescence intensity variations are proportional to the concentration of dissolved acids. In addition, the luminescence properties of other organic molecules such as proteins, permit the qualitative analysis of the composition of the peat extract. These luminescence applications contrast strongly with conventional humification analyses. These examine the absorption/transmission of light at 540 nm, a single point on the tail of an organic acid absorption curve, and hence provide a relatively poor resolution signal of organic acid structure and concentration. In this study we utilize luminescence technology to help understand past peat humification changes.

Approach and methods

Detailed palaeohydrological analyses have been carried out on a number of peat profiles from a raised bog at Derryville, Co. Tipperary, Ireland (52°50′N 7°40′W) as part of the Lisheen Archaeological project (Caseldine *et al.*, 2000). Drainage of the bog for peat extraction for power generation by Bord na Mona, in advance of further destruction by Minorco-Lisheen for a zinc mine, provided peat faces for sampling, although they were partly truncated by peat milling. Coupled with extensive peat-stratigraphic work by Casparie (2000), the palaeohydrological analyses, involving analysis of testate amoebae and water table reconstruction, macrofossil, pollen and spore analysis, and peat humification, has enabled a very detailed understanding of the development of the bog from 7000 cal. BC to cal. AD 1000. An example of this work from site Killoran 18 (DER18 West) is presented in Figure 1 and Table 1, one of the most complete profiles examined, which included the section chosen for detailed luminescence studies because of the extreme range of humification (transmission) values observed over a relatively short profile, especially between 30 and 71 cm. This was the greatest humification shift found in any of the profiles examined.

Full details of the entire profile and the techniques adopted are included in Caseldine *et al.* (1999; 2000), and only the details

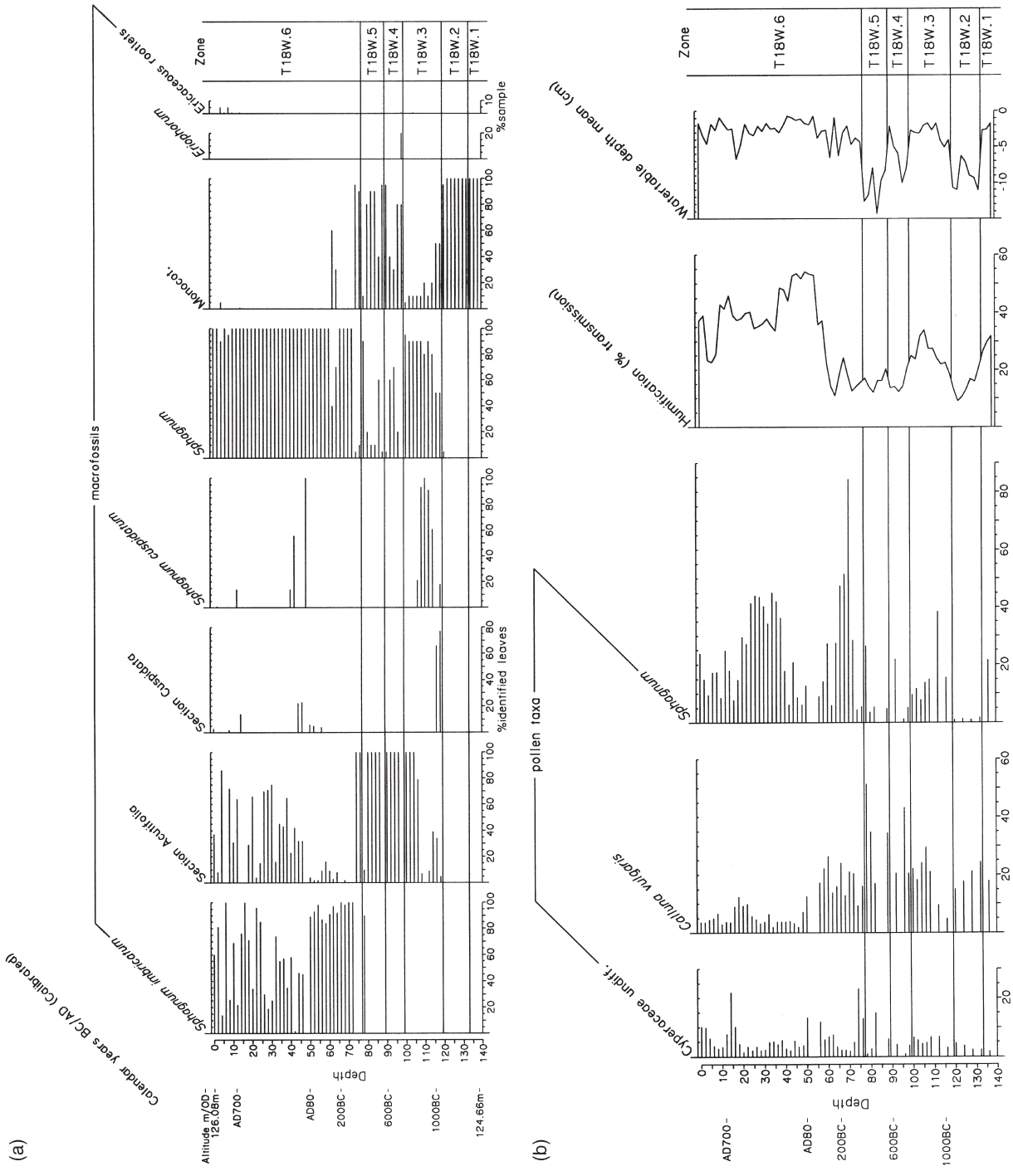

Figure 1 Summary of palaeohydrological indicators for the Killoran 18 profile. (a) Macrofossil record. (b) Pollen of mire taxa; humification expressed as percentage transmission at 540 nm, water table reconstruction based on testate amoebae counts and use of the transfer function of Woodland *et al.* (1998). The dates are based on radiocarbon dating of local pollen zone boundaries from a series of sites across the Derryville Bog (Caseldine *et al.*, 1999), and have been calibrated to incorporate dendrochronologically dated levels. (In this case a wooden trackway which occurs below the analysed profile at 140 cm; see Table 1.) The zones defined on the figure are palaeohydrological zones based on a combination of the proxies used.

Table 1 Field stratigraphy of the Killoran 18 profile, Derryville, Co. Tipperary, Ireland

Depth (cm)	Profile
0–40	Poorly humified *Sphagnum* peat
40–53	Poor humified *Sphagnum* with remains of *Sphagnum cuspidatum*
53–76	Moderately to well-humified *Sphagnum* peat with occasional *Eriophorum vaginatum* stems and *Calluna* twigs
76–98	Moderately to well-humified *Sphagnum* peat
98–120	Moderately humified *Sphagnum* peat with inclusions of *Sphagnum cuspidatum*
120–133	Transitional to fen peat
133–140	Fen peat with rhizomes of *Phragmites australis*
140	Wooden elements of wood and stone causeway Killoran 18 (dated by dendrochronology to 1440 ± 9 BC; Q-9349)

relevant to this paper are presented here. Samples were prepared for testate amoebae analysis using standard methods (Hendon and Charman, 1997), and the curve for depth to water table was reconstructed using the transfer function of Woodland *et al*. (1998). Macrofossil analyses were based on examination of the sediment samples from which the testate amoebae and pollen were extracted, rather than full analyses (cf. Barber *et al.*, 1994), in order to give a general indication of peat composition. Samples for these proxies were taken at 2 cm intervals. The relative proportions of major peat constituents was assessed and expressed as a percentage of the total peat. Assessment of the relative proportion of Unidentified Organic Matter (UOM) was not attempted. An estimate of the *Sphagnum* composition was made by extracting a sample of >50 leaves and counting the leaves of each major Section or species. The leaf counts are expressed as a percentage of the total *Sphagnum*.

Methods for humification determinations follow Blackford and Chambers (1993) and were carried out on 0.5 cm contiguous samples of peat from the monolith. Measurements were undertaken on a Unicam 5625 UV/VIS Spectrometer at 540 nm, and results expressed as 'raw' percentage light transmission values through the peat. Although the measurement process is determining the amount of absorption of light, researchers vary in how such data are presented, with some converting to humification by relating to a humic acid standard, as well as showing the original results as percentage transmission rather than absorption (Chambers *et al.*, 1997). The detailed humification diagram is presented in Figure 2, and shows a very wide range of humification, ranging from very poorly humified *Sphagnum* peat (transmission up to 70%) to very well-humified peat (as low as 5% transmission).

Luminescence measurements on the humification extracts were carried out immediately, or after two months following immediate freezing in 30 ml dark amber glassware. Measurement of luminescence characteristics on the same samples before and after freezing revealed no effects of freezing and storage. The luminescence intensity of the humification extracts was run on samples that had been diluted by ×20. This was done for a number of reasons: (i) to eliminate any interference from absorption effects (Mobed *et al*., 1996); (ii) to lower the pH to a constant value of ~10, as pH affects both luminescence intensity and wavelengths (Miano *et al.*, 1988; Senesi *et al.*, 1991; Mobed *et al.* 1996); and (iii) to decrease the signal to within the range detectable by the spectrophotometer.

Luminescence properties were determined using a Perkin-Elmer Luminescence Spectrophotometer LS-50B, with a Xenon excitation source, and slits set at 5 nm for both excitation and emission. For all samples excitation wavelengths were scanned from 260 to 460 nm at 5 nm steps, with emission wavelengths scanned from 390 to 560 nm at 0.5 nm steps. High-resolution analysis of the luminescence peak at the 290–340 nm excitation, 395–430 nm emission wavelength pair (the location of the 'FA' signal) was also undertaken. In addition, to assess the number and location of luminescence centres, a subsample of extracts representative of each humification and macrofossil type was analysed over a wider range of wavelengths: from excitation wavelengths of 200 to 500 nm at 10 nm steps; for each excitation wavelength, luminescence emission was detected from 220 to 550 nm at 0.5 nm steps.

For each humification extract, the luminescence was determined as the maximum intensity at an excitation-emission wavelength pair. Both the luminescence intensity and emission wavelength of this peak was recorded. Analyses were performed at a laboratory temperature of 22 ± 2°C. Random duplicate samples were run throughout the analysis period; the wavelength of peak luminescence was reproduced within ±3 nm for all analyses and the intensity of luminescence within ±10%. In addition, stability of the Raman peak of a dissolved water blank was assessed for a five-minute period at the start of each day of data collection, and between every 20 samples, in order to assess machine stability. (The Raman peak is the energy produced by the vibration of water molecules when excited by a high-energy light source.) Measurements were only taken when the signal:noise ratio of the blank was greater than 500:1.

Luminescence and composition of humification extracts

Three-dimensional plots representative of the excitation-emission for both extremely poorly and well-humified peat are presented in Figure 3. Figure 3c is from 30.0 to 30.5 cm, within the phase of high transmission of light at 540 nm. Several luminescence peaks are visible; that at 320:440 nm ex:em wavelength pair is that ascribed to 'FA'-like organic matter; that at 270:350 nm ex:em pair to protein luminescence; that at 220:440 nm ex:em pair is poorly defined at present. The peak at 220:300 nm has not previously been reported from the soil or environmental literature, but is typical of a small, highly luminescent molecule such as polysaccharides. Figure 3b shows the luminescence properties from the 67.0–67.5 cm extract, within the zone of low transmission of light at 540 nm. All four luminescence peaks are still present and little difference can be observed between the extracts from both low and high transmission samples.

Figures 3b and 3c demonstrate that several luminescent organic materials are present within the humification extracts. The longer, less broken-down organic molecules (e.g., 'FA') are shown to be present in all samples. However, there is no sign of 'HA' luminescence, which typically occurs at higher excitation and emission wavelengths than the fulvic acid (380–440:400–500nm) (Senesi *et al.*, 1991; Mobed *et al*. 1996). Similarly, the luminescence intensities of relatively low molecular weight, soluble organic molecules such as protein, polysaccharides and amino acids, are typically equal to or greater than that of the fulvic acids.

The luminescence data give a visual record for the degree of breakdown of the organic molecules in the NaOH extractions. It suggests that considerable breakdown of the organic structures has occurred, with all high molecular weight 'humic acids' (HA) altered to lower molecular weight compounds. The similarities between Figures 3b and 3c include a dominance of polysaccharide, amino acid and fulvic acid luminescence centres. The difference between Figures 3b and 3c lies only in relatively subtle changes within the protein and polysaccharide luminescence centres. There are no 'HA' peaks present in either sample. This

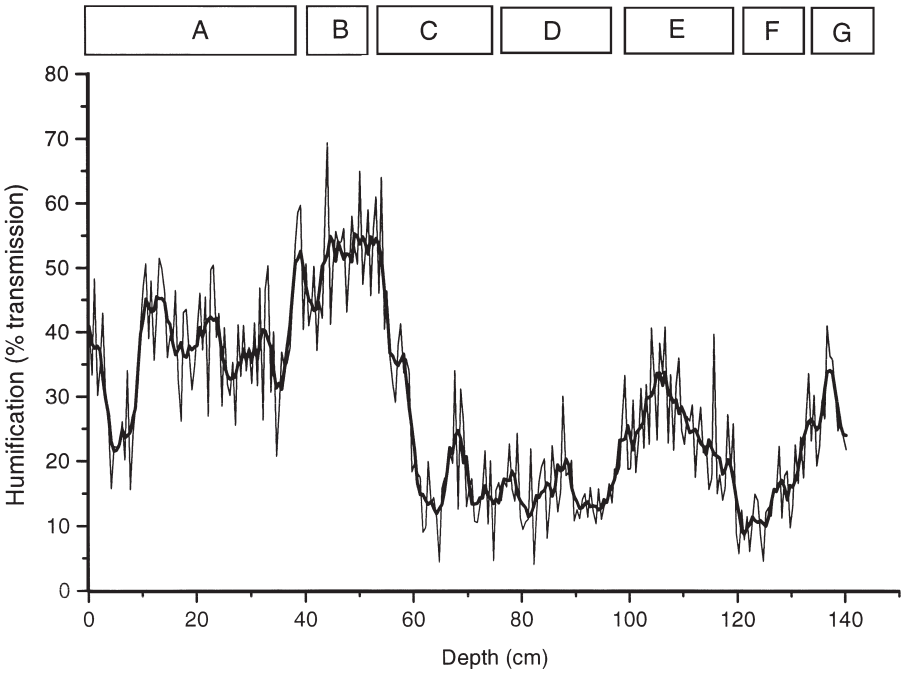

Figure 2 Detailed humification record of the Killoran 18 profile, with three-point smoothing (thick line). Stratigraphy: (A) poorly humified *Sphagnum* peat; (B) poorly humified *Sphagnum* peat with well-preserved remains of *Sphagnum cuspidatum*; (C) moderately to well-humified *Sphagnum* peat with occasional stems of *Eriophorum vaginatum* and twigs of *Calluna vulgaris*; (D) moderately to well-humified *Sphagnum* peat; (E) moderately humified *Sphagnum* peat with inclusions of *Sphagnum cuspidatum*; (F) transition to fen peat; (G) fen peat.

is unlikely to reflect the original composition of the organic acids in the peat, but is more likely to be a product of the NaOH extraction method. During boiling in NaOH, any high molecular weight, aromatic organic acids ('HA') are broken down to lower weight, 'FA', amino acids and sugars. It is possible that these analyses could be influenced by the chemical composition of the bog water contained in the original sample. Although analyses of bog waters can show a range of luminescence characteristics (Baker, unpublished data) they typically have significant proportions of both 'HA' and 'FA', with little protein and no 220:330 nm peak (Figure 3a; note that, because of the absence of an intact bog surface at Derryville, data from Coalburn in the Northern Pennines have been used).

Although the NaOH preparation clearly significantly affects the acid composition of the extracts, it may still be reasonable to assume that, as long as a standard procedure is followed, the degree of extra breakdown caused should remain constant. Thus the measure of humification determined as percentage transmission at 540 nm may enable separation of the relative differences in organic matter decomposition between samples, especially if the amount of initial decay varied significantly. If the NaOH procedure adopted produced differential decay between plant species then the technique should however be seen as unreliable.

Comparisons between luminescence and transmission profiles

Theoretically, measurements of transmission through the extracts at 540 nm are related to the absorption of light that is a function of the molecular weight and concentration of the organic molecules present. As discussed above, emission wavelength is related to molecular weight of the organic molecules and luminescence intensity is a function of the concentration of the molecules related to the particular emission peak. Therefore, transmission should be related to both luminescence wavelength and intensity. Figure 4 shows that emission wavelength of the fulvic acid luminescence intensity maxima has a negative correlation with transmission but luminescence intensity shows no relationship. Regression analyses confirm that transmission is primarily related to luminescence emission wavelength and not intensity (transmission percentage = 502–1.11 LW, $R^2 = 36.7\%$; transmission percentage = 480.2–1.04 LW–0.32 LI, $R^2 = 41.6\%$; where LW = luminescence wavelength and LI = luminescence intensity). This demonstrates that percentage transmission is primarily a function of molecular weight of the organic matter in the extracts. It is, however, not in the expected direction for, as percentage transmission decreases, i.e., as the amount of humification increases, the emission wavelength also increases, representing the effect of more high molecular weight acids (FA).

The most noticeable feature of the luminescence curve is that there is a very sudden change from predominantly high emissions to low emissions at 56.5 cm before relatively stable results over the next 10 cm. This occurs at the middle of a shift from well-humified to poorly humified peat, where the change in transmission characteristics occurs over a greater depth. In a temporal sense, this equates to a luminescence change in under a decade compared to a humification change spanning over a century (61–54 cm, at 16 yr cm^{-1}). Given that the humification and luminescence analyses were performed on the same extracts and are both related to organic acid properties, the relatively rapid response of the luminescence signal with respect to the transmission results reflects the signal:noise ratio of the respective techniques. Transmission at 540 nm is attempting to differentiate optical transmission at the tail of a broad exponential decay curve, thus signal resolution is relatively poor. Luminescence wavelength variations have a significantly higher signal:noise ratio, with well-resolved luminescence centres clearly differentiated.

There are difficulties in comparing directly the luminescence and transmission data with the other palaeohydrological proxies owing to a coarser sample resolution in the latter (2 cm as against 0.5 cm), but the sudden change in luminescence wavelength occurs at a slight change in peat composition with the introduction of *Sphagnum* Sect. Cuspidata (Figure 1). This change is, however, small and the macrofossil content of the peat is still exclusively

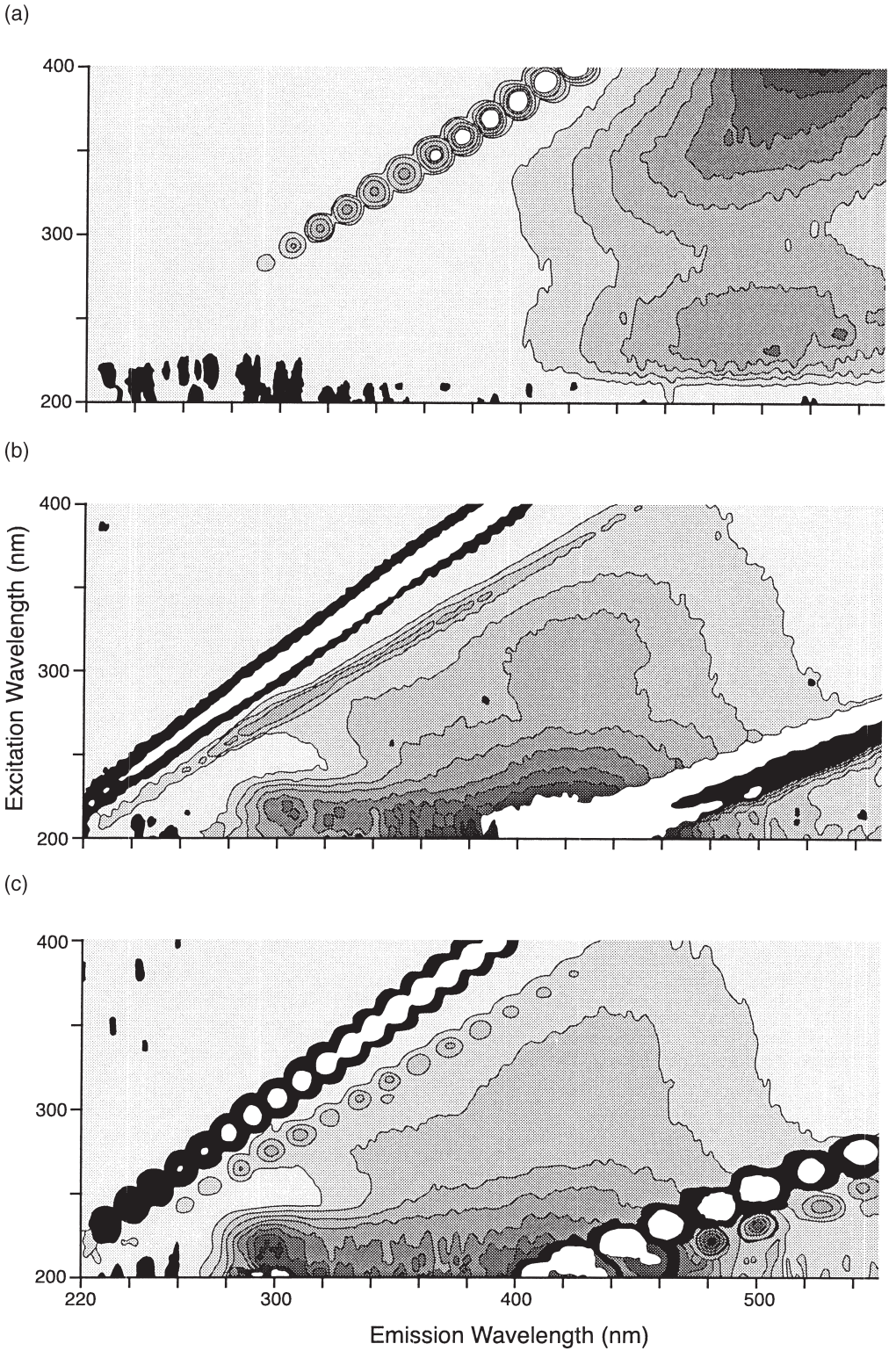

Figure 3 Three-dimensional plots of excitation-emission wavelength and intensity for: (a) typical bog water (from the Coalburn Experimental Catchment, Northern Pennines; (b) 67–67.5 cm, a high-humification, low-percentage transmission sample; (c) 30–30.5 cm, a low-humification, high-percentage transmission sample. Expected peak locations for the excitation-emission plots are: HA 250:480 and 400:480 nm; FA 300:410 nm; protein 275:350 nm; sugar – uncertain but thought to be 220:300 nm. The linear features are Rayleigh scatter lines due to reflection off the water surface of the samples, and are thus an artifact of the machine. The actual intensities vary from sample to sample, but cannot be compared directly, hence it is the location and character of the observed luminescence centres that is significant. The bog water clearly shows the HA centres with little signal for sugars or proteins, whereas the two 'humification' samples are remarkably similar, have no HA, a more or less well-defined FA centre, and are predominantly sugars and proteins.

Sphagnum, predominantly *S. imbricatum*. Thus, although some workers have shown differences in susceptibility to decay of different plant groups or *Sphagnum* taxa (Johnson *et al.*, 1990; Johnson and Damman, 1991; Lehtonen and Ketola, 1993), it seems unlikely that the species change seen here could account for such a shift in the luminescence data. Variable susceptibility to decay within species, could, however have a potential effect on the degree to which they are affected by the NaOH treatment, with those *Sphagnum* species showing least resistance to decay giving an apparently greater 'humification' signal after the same length of time in NaOH. Comparison of the luminescence signal with the testate amoebae record on which the water table reconstruction

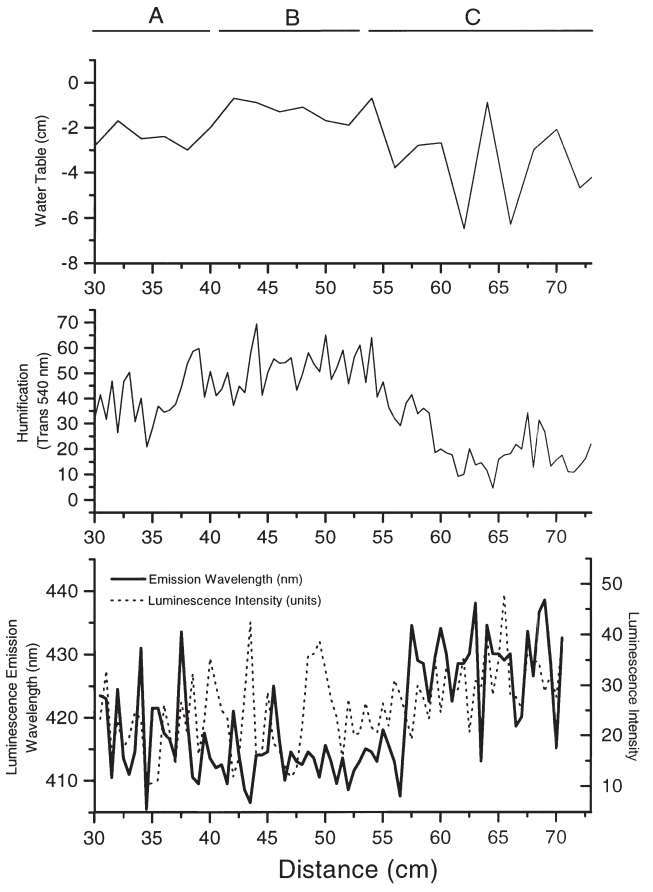

Figure 4 Summary of palaeohydrological proxy data compared with luminescence properties for 30–72 cm in the Killoran 18 profile: Top: reconstructed water table based on testate amoebae analysis. Centre: humification measured by transmission at 540 nm, high values represent high transmission and therefore low humification. Bottom: luminescence emission wavelength and intensity of the fulvic acid luminescence intensity maxima. The stratigraphy is summarized as A–D; see Figure 2 for explanation.

(Figure 5) is based shows a close association with the shift from *Arcella discoides* type to *Amphitrema wrightianum*, and overall the period of lower emission wavelength mirrors closely the presence of this taxon, and the absence of *Hyalosphenia subflava*, together indicative of a particularly wet peat surface. A possible explanation of the association of the two records would be to see both these taxa and the luminescence signal as particularly sensitive indicators of changing surface hydrology, while the use of assemblages tends to smooth the record. This could have important implications for the way we approach records, i.e., whether an assemblage or an indicator species approach may be most suitable. The present study is, however, very much a preliminary empirical analysis which, while showing considerable potential, has emphasized areas that require more wide-ranging and rigorous examination.

A further factor which could affect the luminescence response, and one which may prove more difficult to overcome, is in the amount and origin of the UOM in samples. This is by no means restricted to the luminescence signal as it also affects the transmission-driven 'humification' data. By definition UOM may have a wide range of sources, including material such as lichens rarely discernible in macrofossil analysis. In their examination of biomarkers in ombrotrophic peat Ficken *et al.* (1998: 235) when looking at lipid content argue that 'lichens and other [than *Sphagnum*] species may also have made a greater contribution than is indicated by the macrofossil analysis due to the rapid breakdown in the peat'. Quantitative estimates of UOM tend to be highly

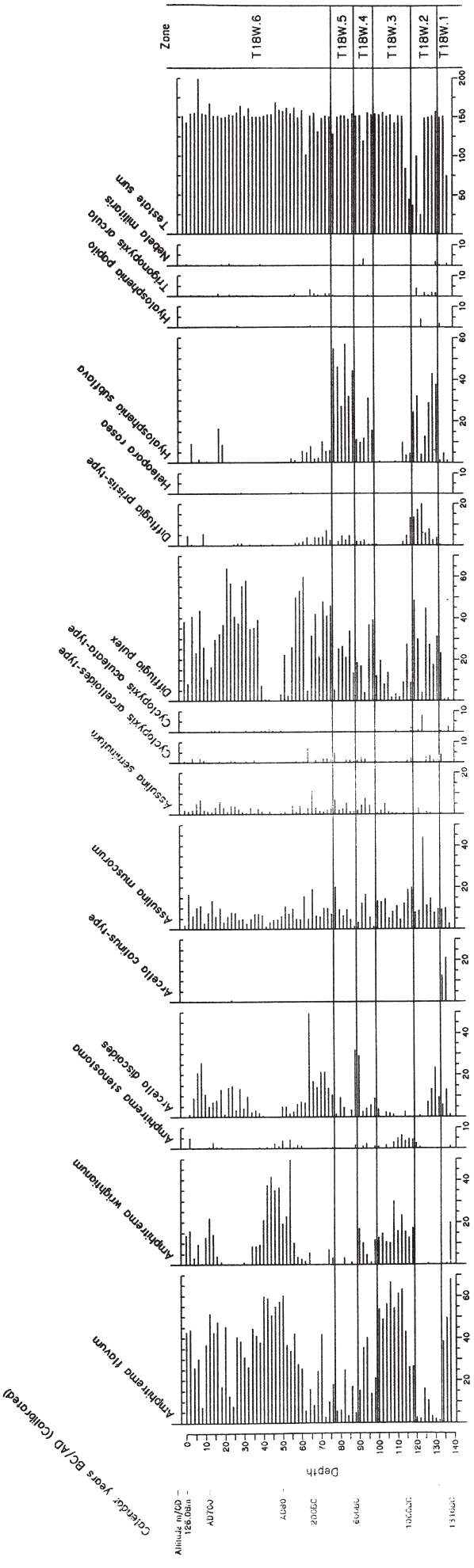

Figure 5 Testate amoebae data from Killoran 18 (DER 18 WEST). Zones are palaeohydrological zones defined on the basis of all the palaeohydrological proxies.

subjective and difficult to correlate across profiles, but if it was the main effect behind the chemical changes registered in humification analysis there should be a consistent relationship whereby high estimates of UOM correlate with high humification and low light transmission. Examination of detailed analyses from the recent literature (e.g., Barber *et al.*, 1998; Charman *et al.*, 1999; Mauquoy and Barber, 1999) shows that such a consistent pattern cannot be found. Nevertheless, the potential contribution of changes in the sources of UOM in affecting luminescence and humification responses remains a problem, one which may perhaps only be solved by successful NMR fingerprinting (McTiernan *et al.*, 1998).

Thus as far as the luminescence emission data are concerned there is a statistically significant, but weak, relationship between humification and maximum emission, but with higher emissions in general reflecting greater levels of humification (lower percentage transmission). This is in contrast to the underlying principle of colorimetric determination of 'humification' whereby transmission levels decrease with increasing plant breakdown. Transmission does not measure the amount of humic acids, but is a response to the relative amounts of 'FA', amino acid, protein and polysaccharide structures present in the NaOH extracts. Luminescence intensity shows no clear relationship with transmission, and exhibits a ×3 variation from ~15 to ~50 units, demonstrating a considerable variability in the extent of 'FA' breakdown during the 'humification' procedure. Extracts with high 'FA' luminescence intensities again suggest a more limited degradation during the NaOH treatment. Given that differential decay rates between bog species are relatively well known (Coulson and Butterfield, 1978), with field experiments for instance demonstrating the relatively slow decay of *Sphagnum fuscum* and *Racomitrium lanuginosum* as compared to *Sphagnum cuspidatum* (Johnson *et al.*, 1990; Johnson and Damman, 1991; Belyea, 1996), it is likely that changes in species at the bog surface would influence 'natural' rates of humification, as commented upon by Chambers *et al.* (1997), but also that there will be a similar differential susceptibility to the NaOH treatment. Hence the presence of relatively more resistant species would enhance FA intensities. The close relationship between curves for certain testate amoebae species and luminescence wavelength changes also possibly points to the importance of microfaunal effects on peat transformation as represented by the 'humification' signal. Clearly further work on a range of peat types with higher-resolution macrofossil and testate amoebae data is required to examine what the luminescence wavelength and intensity signal actually represents, and whether the colorimetric measurement of peat 'humification' represents a proxy signal which can be related with confidence to climate. The empirical evidence presented here underlines the weakness of our understanding of the processes involved in producing such a signal.

In summary, the results from the luminescence analyses across this extreme range in humification do suggest some correlation between colorimetrically measured 'humification' and luminescence, but this is of a very limited nature. The NaOH procedure produces a mix of organic decay products that reflect a series of factors, probably including the concentration and relative proportions of 'HA' and 'FA' in the original peat, but also species composition and susceptibility to further breakdown by the procedure itself. The procedure is also sufficiently effective to remove all HA present in the original sample. Luminescence analysis is able to identify changes in these parameters and provides a method for beginning to unravel the processes involved in peat decay and changes in chemical composition related to the palaeohydrology of the mire surface, particularly if procedures can be applied which have less of an impact on the structure of acids within the peat. Relative peat humification along a profile measured by the standard colorimetric procedure should therefore be seen as a reliable indicator only of major shifts in surface wetness which can lead to considerable change in the decay regime of the bog surface. Less radical changes may mainly represent noise in the humification signal and should not necessarily be interpreted as indicative of significant shifts in surface conditions. Links therefore to external forcing of such conditions should be inferred with great care, unless supported by a range of other proxies. This finding echoes concerns expressed by Ficken *et al.* (1998) from an ombrotrophic peat profile at Moine Mhor in the Cairngorms, who were able to show only limited agreement between macrofossil analyses and lipid biomarker analyses. Our understanding of the chemical background to the formation of deposits in peat bogs used to provide palaeoclimatic proxies is still relatively poor, but luminescence analysis provides a potentially valuable approach to improving our understanding.

Conclusions and future prospects

Determination of humification changes through treatment in NaOH alters the organic matter present in the undisturbed peat. The inferred presence of enhanced levels of amino acids, protein and polysaccharide groups in humification extracts suggests that a significant proportion of the organic matter is degraded from high to low molecular weight organic acids, and finally evolved as gases (CO_2, etc), and that no 'humic acids' (HA) are present after treatment. As long as the procedure is applied rigorously it may still provide an environmental proxy, but it is measuring results of processes that are poorly understood, and can be influenced by changes in plant species.

One of the main problems of multi-proxy studies of ombrotrophic peats looking for climatic signals is that the differing proxies, macrofossils, testate amoebae and humification rarely contribute a consistent record, tending only to agree at wet or dry extremes. In the absence of rigorous climate transfer functions, or a full understanding of the understanding of the processes involved, as is the particular case with humification, it is difficult to know how best to interpret these differences. While the addition of luminescence spectroscopic analyses, by adding a further potential proxy, adds to this problem of which record is the more sensitive, and hence reliable indicator of climatic forcing, it potentially provides greater insight into the actual processes being observed.

Luminescence spectroscopy is potentially an excellent tool with which to assess the nature of the humification signal and influences such as species-driven decay. Ultimately, it may provide detailed data on the degree and nature of peat decay. Future research using luminescence in peats needs to focus on developing less destructive extraction techniques that do not affect the acid structure to the same degree, or on fibre-optic-based technology that is non-destructive.

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