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Influence of source vegetation and redox conditions on lignin-based decomposition proxies in graminoid-dominated ombrotrophic peat (Penido Vello, NW Spain)

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Keywords

Peatland; Pyrolysis-GC-MS; Lignin degradation; C/N ratio; Graminoids; Ericoids.

1. Introduction

Ombrotrophic peatlands receive water and nutrients from precipitation alone. Although autogenic processes may influence the water table (Malmer et al., 1994), the depth of the water table within a given peatland is to a large extent dependent on precipitation. Water table depth determines oxygen availability and thereby it controls peat decomposition (Abbott et al., 2013; Philben et al., 2013). The degree of decomposition is therefore often used either as a proxy for climate (Blackford and Chambers, 1993), indicating the relative importance of drier and wetter periods, or to obtain information on the rate of organic carbon sequestration (Clymo et al., 1998).

Lignin is a major component of plant remains in peatlands, due to slow decomposition rates under anoxic conditions (Kirk and Farrel, 1987; Williams and Yavitt, 2003). Lignin is a macromolecule composed of syringyl (S), guaiacyl (G) and *p*-hydroxyphenyl (H) sub-units that are irregularly bound to each other. Its composition differs between major plant groups. In addition, non-woody tissue possesses, besides lignin, a high content of free and bound phenols that are ester- and/or ether-linked to lignin and polysaccharides in the cell wall (Hedges and Mann, 1979; Kondo et al., 1989; Lam et al., 1992; Sun et al., 2011). Apart from these source characteristics, the composition of lignin moieties can be influenced by side chain oxidation and shortening during decay (e.g. Kögel-Knabner, 2002). Thus, the composition of lignin in peat contains information about plant source and degradation state that may provide useful information about past environmental conditions (Kuder and Krüge, 1998; Bourdon et al., 2000; Zaccone et al., 2008; Disnar et al., 2008).

Knowledge of lignin transformation in soils and sediments is based mainly on aerobic decay (Thevenot et al., 2010) and woody tissue (Donaldson, 2001), while lignin research in graminoids has concentrated on forage quality (Buranov and Mazza, 2008). The effects of decomposition on graminoid tissue in peat have been studied by few authors (Kuder et al., 1998). Although anaerobic degradation of lignin proceeds at a slower rate (Opsahl and Benner, 1995) and lignocellulose can be completely degraded under anaerobic conditions (Benner et al., 1984), information about the pathways of anaerobic lignin degradation is scarce (Young and Frazer, 1987; van der Heijden and Boon, 1994). The effect of decay on the original lignin plant signal in environmental archives has been reviewed recently by Jex et al. (2014), who conclude that the interpretation of lignin proxy records demands a thorough understanding of the many processes that may be involved. In contrast, it has also been found that plant material affects the interpretation of lignin decomposition proxies in soils (Nierop and Filley, 2007; Mason et al., 2009). Because lignin composition varies between plant parts and elements of plant cells (Grabber et al., 2004), its resistance to decay may show a similar variation (van der Heijden et al., 1994; Williams and Yavitt, 2003; Machinet et al., 2011). Thus, in peatlands, with prevailing anaerobic conditions and significant contribution from graminoids, interpretation of lignin characteristics is intricate. Environmental interpretation of proxy records in peatlands is further complicated by the fact that changes in hydrology drive changes in both plant species composition and the nature and degree of decomposition (Yeloff and Mauquoy, 2006).

Pyrolysis-gas chromatography-mass spectrometry (pyrolysis-GC-MS) is applied frequently to study lignocellulosic material (Ralph and Hatfield, 1991; Meier et al., 1992). In addition, pyrolysis-GC-MS provides detailed information on the overall molecular composition, which benefits the interpretation of complex mixtures of

organic matter in peatlands and soils. The Penido Vello peat (Galicia, NW Spain) is dominated by graminoids, with significant contributions from ericoids at some depths. A high-resolution sampled peat core in this bog has been previously studied with pyrolysis-GC-MS. Schellekens et al. (2011) combined depth records of biomarkers of peatland plants with present-day plant ecology to reconstruct bog hydrology. Assigned wet and dry periods agreed well with other European studies. Thus, the Penido Vello bog, with its dominance of graminoids and known past vegetation shifts, is particularly suited to study the effects of decomposition on the lignin composition in peat.

To separate the effects of both source material and decomposition processes, a selection of fifteen samples from the same core were previously studied in detail by Schellekens et al. (2012). Analysis of peatland plants and comparison of NaOH-extractable organic matter (reflecting decomposition) and the non-extractable residue (reflecting source vegetation; Buurman et al., 2006) allowed separation of source effects (graminoid vs. ericoid material) and different decay processes on the peat lignin composition. The results indicated that during aerobic decay at the bog surface non-lignin phenolics are rapidly lost, and alkyl side chain reduction occurred for G moieties only. Depth related lignin decay (long term anaerobic decay) caused oxidation and reduction of alkyl side chains. Sub-surface (secondary) aerobic decay during water table drawdowns caused fragmentation of lignin while lignified cellulose was less affected. The effects were sometimes contrasting, for example G moieties were preferentially degraded during aerobic decay at the surface while S moieties were preferentially degraded during sub-surface aerobic decay. The results by Schellekens et al. (2012) were based on pyrolysates from two peat fractions that were not quantified. Because analytical pyrolysis provides relative instead of absolute abundances and because

relatively few samples were used, it remains unclear to which extent the results can be generalised and applied to bulk samples.

In order to evaluate the net effect of environmental factors, the present study applied a number of lignin-based parameters, extracted from Schellekens et al. (2012) to pyrolysates of 51 bulk samples from the upper meter of the Penido Vello peat core. To support the pyrolysis results, C/N and ^{13}C CPMAS NMR data were used. The purpose was to (i) identify and separate the effects of botanical shifts and several decomposition stages (aerobic and anaerobic) on the lignin composition in bulk samples, and (ii) examine the use of lignin-based decomposition proxies as a tool for (palaeo)climatic interpretation.

2. Methods

2.1. Location and sampling

Penido Vello is an ombrotrophic mire in the Xistral Mountains (Galicia, NW Spain). Location, sampling and characteristics of the bog have been described in detail by Martínez-Cortizas et al. (1997, 2002). The 3 m deep core dates back to 6000 yr BC. For this study, only the samples from the upper 1 m were used (51 continuous samples of 2 cm in thickness), as this section showed better correlation between vegetation markers than the deeper part (Schellekens et al., 2011), which had been sectioned into 5 cm slices. It represents ca. 2000 yr of peat accumulation. Samples were dried at 35 °C (1 week) and ground for pyrolysis without further treatment.

2.2. Vegetation shifts and characteristics in the Penido Vello peat record

The ombrotrophic section of the peat core was dominated by the graminoids, *Carex durieui*, *Agrostis curtisii*, *Molinia caerulea*, with significant contributions from ericoids

(*Erica mackaiana* and *Calluna vulgaris*), *Eriophorum angustifolium*, *Festuca rubra* and mosses at some depths. Although *Sphagnum* was present in most samples its contribution to the peat biomass was low. Major vegetation shifts upon changes in hydrology have been found between *C. durieui*, *A. curtisii*, *F. rubra*, *E. mackaiana* and *C. vulgaris* (drier conditions) and *M. caerulea*, *E. angustifolium* and moss (wetter conditions; Fraga et al., 2005).

The contribution of roots to the chemistry of older peat layers may complicate a correct palaeohydrological interpretation. Plant morphology, growth characteristics, and good correlations between depth records of peatland plant biomarkers with those of decomposition proxies, indicate that such root effects are negligible for Penido Vello. This aspect is discussed in detail by Schellekens et al. (2011). It is assumed that a loss of lignin phenols as dissolved organic matter is negligible because the peat is located on the summit of a mountain, without any evidence of an outlet.

2.3. Selection of lignin parameters

Based on the results of the preliminary study by Schellekens et al. (2012), a number of lignin-based decomposition parameters were extracted. They are given in Table 1 and structure formulae of associated lignin pyrolysis products are given in Fig. 1.

2.4. Total carbon and nitrogen

The samples were analysed for total carbon and total nitrogen, as described by Pontevedra-Pombal et al. (2004).

2.5. Solid state ^{13}C CPMAS NMR spectroscopy

The Penido Vello core was previously analysed with ^{13}C CPMAS NMR, mean values for each stratigraphic layer (Pontevedra-Pombal et al., 2001) and depth records of the whole core (Pontevedra-Pombal et al., 2004) are already published. The ^{13}C CPMAS NMR spectra were divided into chemical shift regions: 0–46 ppm, aliphatics; 46–60 ppm, methoxyl; 60–95 ppm, O-alkyl C; 95–110 ppm, acetyl; 110–140 ppm, aromatics; 140–160 ppm, phenolics; and 160–250 ppm, carboxyl (Pontevedra-Pombal et al., 2001; Kaal et al., 2007). For a detailed description of the methodology we refer to these studies.

2.6. Pyrolysis-GC-MS

A Curie-Point pyrolyser (Curie temperature 600 °C) connected to a Carlo Erba GC8000 gas chromatograph was used. Pyrolysis products were separated on a non-polar fused silica column [Chrompack: 25 m, 0.25 mm i.d., CP-Sil 51 b (0.40 μm)], with He as carrier gas. The oven temperature programme was: 40 °C to 320 °C (held 15 min) at 7 °C min^{-1} . The column was connected to a Fisons MD800 mass spectrometer (m/z 45–650, cycle time 1 s).

Quantification in the present study was identical to that of Schellekens et al. (2012; Appendix) and based on the peak area of two major fragment ions from each pyrolysis product. All individual quantifications were checked manually. For each sample, the sum of the peak areas was set at 100 % and proportions were calculated relative to this. According to probable origin and similarity, the pyrolysis products were grouped as follows: polysaccharides, aliphatic hydrocarbons, methyl ketones, lignin products, phenols, catechols, (other) aromatic hydrocarbons, polyaromatic hydrocarbons, nitrogen containing compounds, fatty acids, steroids and triterpenoids (Appendix).

2.7. Statistical analysis

In order to identify and separate the effects of the main environmental factors/processes influencing peat lignin composition of the 51 analysed samples, factor analysis was applied using 120 quantified pyrolysis products (Appendix; Schellekens et al., 2012) and 15 lignin parameters (Table 1; Section 2.3). The identification of the factors was done using the explained variance of the pyrolysis products as reflected by the factor loadings. Thereafter, the contribution of the factors to the variation in lignin parameters was determined. The factor scores were used as a measure of the effect/weight of the factor on the composition of any given sample and allow comparison with other characteristics such as ^{13}C NMR and C/N. Factor analysis was performed with Statistica® Version 6 (Statsoft Inc, Tulsa). All correlation coefficients had a significance level of $P < 0.00001$.

3. Results and Discussion

The identification of the effects of environmental factors on the lignin parameters is described in Section 3.1; the net effect underlying the extracted factors is discussed according to depth profiles of the lignin parameters in Section 3.2, and their palaeohydrological value is discussed in Section 3.3.

3.1. Factor analysis

The first four extracted factors explained the major part of the total variance (69.3%). Projections of the loadings of factors 1 and 2 (F1, F2) and factors 3 and 4 (F3, F4) are given in Fig. 2. F1 (36.3% explained variance) is mainly characterised by positive loadings of lignin phenols and short to mid-chain *n*-alkanes (C_{10-25}) and negative loadings of polysaccharide pyrolysis products and long chain *n*-alkanes (C_{29-33} ;

Fig. 2a). In peat, polysaccharides are preferentially degraded over lignin (Benner et al., 1984; van der Heijden et al., 1990; Disnar et al., 2008) and an increase in aerobic decay causes chain length reduction of *n*-alkanes (Eglinton and Hamilton, 1967; Schellekens and Buurman, 2011). F1 is therefore assigned as reflecting bog hydrology at the time of peat formation and thus the first stage of decay.

The high positive loading of aliphatic products on F2 (12.9% explained variance; Fig. 2a) indicated residual accumulation of these compounds upon diagenesis, as an increase in aliphatics was found for the whole (3 m) peat record with ¹³C CPMAS NMR (Pontevedra-Pombal et al., 2001; Buurman et al., 2006) and pyrolysis-GC-MS (Schellekens et al., 2011). This indicates that F2 reflects long term anaerobic decay.

F3 (11.5% explained variance) showed high negative loadings for a sterol derived (S3) and an aliphatic (Al2) pyrolysis product, that were obtained solely from ericoids and woody plants, respectively (Schellekens et al., 2011), and long chain *n*-alkanes (Schellekens and Buurman, 2011). Compounds with positive loadings on F3 were associated with lignocellulose of graminoids: 4-vinylguaiacol from ferulic acid (Lg12) and 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one (Ps4; Section 3.2.4). Therefore, F3 separates ericoids (negative loadings) from graminoids (positive loadings, Fig. 2b).

F4 (8.6% explained variance) showed positive loadings for lignin products with a C₃ alkyl side chain that reflect unaltered lignin (Lg13, Lg16, Lg17, Lg31, Lg33, Lg37; van der Hage, 1993; Kuder and Krueger, 1998), while benzene (Ar1), toluene (Ar2), pyridine (N1) and polyaromatic products (PA1, PA2) showed negative loadings and are associated with aerobic decay (Schellekens et al., 2009). F4 is therefore assigned as reflecting secondary aerobic decay that occurs due to events of lowering of the water table. Positive loadings on F4 indicate relatively undecomposed material (Fig. 2b) and thus high water table during the development of the layer.

The first factor extracted showed significant correlation with data obtained from ^{13}C CPMAS NMR (Fig. 3; Table 2): negative correlation of O-alkyl C (r^2 0.82) and acetal C (r^2 0.66) was found with F1, while aromatic C (r^2 0.86) and phenolic C (r^2 0.77) were positively correlated with F1, reflecting preferential decay of polysaccharides over lignin. The agreement between pyrolysis and ^{13}C CPMAS NMR data indicates that the trends obtained with analytical pyrolysis are valid despite the semi-quantitative character of the method (see Section 2.6).

The identified factors (F1–F4) that influenced peat chemistry are summarised in Table 3. During the first stage of decay at the surface, aerobic (water table below the surface) and anaerobic conditions (water table at or above surface) altered the organic matter differently. In the sub-surface, secondary aerobic decay may occur when the water table lowers during drier phases. Although the degree of decay reflects a continuum, primary and secondary aerobic decay showed different effects on peat chemistry. Once in the anaerobic zone (catotelm), the peat is subject to long-term decay. The variance of each individual lignin parameter accounted for by the four factors is reflected by the fractionation of communalities (Fig. 4). Communalities for the remaining factors (F5–F16) and individual pyrolysis products are not shown.

3.2. Effects of vegetation composition and decay processes on the lignin parameters

3.2.1. Abundance of lignin and polysaccharides (parameter 1)

The summed lignin (parameter 1a) and summed polysaccharide (parameter 1b) pyrolysis products were for > 80% explained by F1 (Fig. 4), reflecting bog hydrology at the time of peat formation (Table 3). High values of parameters 1a and 1b indicate relatively dry and wet periods, respectively (Fig. 2), and reflect the preferential decay of polysaccharides over lignin during the first stage of decay. The remaining variance of

polysaccharides (parameter 1b) was explained by F2 and F3 (Fig. 4) and is discussed in Section 3.2.4. The remaining variance in lignin (parameter 1a) was explained by F2 (Fig. 4) and is predominantly related to G moieties (Fig. 2a; Section 3.2.2.1).

3.2.2. Preferential decay of lignin moieties (parameter 2)

3.2.2.1. Syringyl to guaiacyl ratio

The analysis of different peat fractions indicated that differentiation between S and G moieties (parameter 2a) was predominantly related to decomposition instead of vegetation shifts (Schellekens et al., 2012). To exclude a contribution from non-lignin phenolic acids to the abundance of lignin moieties, only the ratio of S to G units with a C₃ alkyl side chain was chosen (parameter 2b), as these lignin moieties reflect macromolecular lignin (Kuroda, 2000; van der Hage et al., 1993). Demethoxylation during pyrolysis is negligible (Harman-Ware et al., 2013) and can be excluded.

Both S/G ratios, parameters 2a and 2b, were explained for > 85% by the first four factors, of which the major part was related to F1 (Fig. 4). The projection of parameters 2a and 2b was negative on both factors F1 and F2 (Fig. 2a), and their depth profiles showed an increase until 80 cm from where they stabilised (Fig. 5). This pattern indicated preferential decay of G moieties, both during primary decay at the surface (F1) and during long term anaerobic decay (F2). For parameter 2a an additional part of the variance was explained by F4, reflecting secondary aerobic decay in the sub-surface (Fig. 4). Parameter 2a de-trended for F1 and F2 (i.e. eliminating the effects of F1 and F2) showed a good positive correlation with F4 (r^2 0.63, not shown), indicating that, during secondary aerobic decay, S moieties were preferentially degraded over G moieties. The very low explained variance on F4 for parameter 2b (Fig. 4) indicates that

preferential decay of S moieties is not valid for lignin C₃ alkyl side chains (see also Section 3.2.3).

The preferential decay of G moieties was attributed by Schellekens et al. (2012) to a high contribution from a non-lignin phenolic acid source and thus not from macromolecular lignin. An increase in S/G ratio under anaerobic conditions was also found for graminoid tissue by Opsahl and Benner (1995) and in graminoid-dominated peat by Kuder et al. (1998). Preferential decay of S lignin, related to its structure, is generally described from aerobic systems (Thevenot et al., 2010) except during the earliest stages of decay (Christman and Oglesby, 1971; Kögel, 1986). Because anaerobic conditions predominate in peat, this first stage of decay is prolonged, and the results thus correspond well with current knowledge. However, because moieties with a C₃ alkyl side chains originate from the lignin macromolecule and parameters 2a and 2b showed similar behaviour in factor analysis, an additional explanation may be that of differential decay of plant tissues. Because structure and chemical composition of plant material may differ greatly within both plant parts and plant cells (Buranov and Mazza, 2008), it is likely that decay is connected to the anatomical structure of the plant, and thus to the accessibility of S or G lignin. This is in agreement with Grabber et al. (1997, 2004) whose results indicate that the degradability of grass cell walls was not affected by the composition of lignin moieties.

The graminoid species that dominate the Penido Vello peat have similar morphology, with thin leaves and a rigid stem (Schellekens et al., 2011), which suggests that the leaves are more easily degradable than the stems. According to macrofossil analysis of a similar peat record located at 2 km distance (Pena da Cadela), the peat is dominated by roots, rhizomes and internodes of graminoids. Remains of epidermis (which may originate from both leaves and stems) are also abundant but are much

smaller in size and fragmented (I. Fraga, personal communication). The preferential decay of leaves vs. other plant organs in the peat may explain (part of) the abundance of G and S lignin during decay. This is supported by lignin studies of other graminoids. In brome grass (Jung and Casler, 1990) and barley (Love et al., 1998), S lignin was found in higher abundance in the stems than in the leaves and was more resistant to decay than G lignin, while the latter had a much higher abundance in the middle lamellae and was more easily decayed (Love et al., 1998). Furthermore, an increase in S/G with maturity in the stem of several graminoids (Grabber et al., 2004) was negatively correlated with enzymatic degradability in *Festuca arundinacea* (Chen et al., 2002). Thus, in addition to preferential decay of G moieties from non-lignin phenolic acids, the observed decrease in G lignin under both aerobic and anaerobic conditions during the first stage of decay (Table 2) is presumably related to plant anatomy. In woody tissue (e.g. from ericoids) the lignin composition and plant architecture is different and thus preferential decay of S moieties in peat in which ericoids determine the lignin content (van der Heijden and Boon, 1994; Huang et al., 1997) does not necessarily contradict to the preferential decay of G moieties in graminoid peat.

The source material (graminoids) therefore exerts a strong influence over 'preferential decay' of G or S moieties in peat. In agreement with this conclusion is the fact that F3, which differentiated graminoids from ericoids, showed negligible explained variance for both S/G ratios (Fig. 4). Thus, for peat dominated by graminoids, parameters 2a and 2b would not be related to *shifts* between graminoids and ericoids, but would be almost completely determined by decay (F1, F2 and F4).

3.2.2.2. *p*-Hydroxyphenyl moieties

Parameter 2c reflects the relative abundance of H moieties within the lignin macromolecule. Lignin moieties with a vinyl side chain (4-vinylphenol Lg1, 4-vinylguaiacol Lg12 and 4-vinylsyringol Lg30) were excluded from this parameter because of their dual origin from both non-lignin phenolic acids and macromolecular lignin (Section 3.2.4).

The first four factors explained only 52% of parameter 2c (Fig. 4). F3, which distinguished graminoids from ericoids, accounted for the largest part of the explained variance. Thus, although demethylation during the first stage of decay and long term anaerobic decay occurs in peat (Tsutuki, 1994; Filley 2003; Schellekens et al., 2012), the influence of such decay (F1, F2) was lower than that of source vegetation. The negative loadings on F3 (Fig. 2b) indicated that H moieties had a relatively higher abundance in the peat samples with a higher contribution of ericoids. This higher abundance in ericoids contradicts the general interpretation of H lignin as being indicative for grass (Hedges and Mann, 1979; Thevenot et al., 2010), and is presumably related to the fact that H moieties may also originate from non-lignin phenolic polymers such as tannins (Wilson et al., 1985), that have a higher abundance in woody species (Kögel-Knabner, 2002).

3.2.3. Side chain oxidation and shortening (parameters 3 and 4)

Side chain oxidation highly increases from peatland plants to peat OM (Schellekens et al., 2012). A commonly used parameter for lignin degradation is the acid/aldehyde ratio (Ac/Ad) of G and S moieties after oxidation with CuO (Ertel and Hedges, 1985; Thevenot et al., 2010). With pyrolysis however, G and S moieties with a C₃ alkyl side chain, parameters 4a and 4b respectively, are generally interpreted as indicating intact lignin (Mulder et al., 1991; van der Heijden and Boon, 1994). For the present samples,

the Ac/Ad ratio was completely determined by the acid moieties (not shown). Therefore, vanillic acid and syringic acid were expressed as percentage of G and S, parameters 3a and 3b, respectively. In addition to the acids, 4-acetylguaiacol and 4-acetylsyringol were chosen to reflect oxidation of lignin alkyl side chains (parameters 3c and 3d, respectively).

Although vanillic and syringic acid were expected to increase upon (secondary) aerobic decay in peat, parameters 3a and 3b had no loadings on F4 and a major part of the observed change was related to undecomposed organic matter (F1) and ericoids (F3; Fig. 4). Low Ac/Ad values that did not correspond to other degradation parameters were also found by Disnar et al. (2008) in a sedge-dominated peat. An explanation may be that a high abundance of acids in graminoid-dominated systems is related to a change in vegetation composition. The knowledge that vanillic and syringic acid result from fungal oxidative cleavage of C₃ alkyl side chains is based on wood lignin (Saiz-Jimenez and de Leeuw, 1984; Hedges et al., 1988; Higuchi, 1990). Parameter 3a showed a negative correlation with F1 only for the samples that had negative scores on F1 (r^2 0.64, $n = 22$; not shown). For these samples, vanillic acid may have originated from non-lignin phenolic monomers known to be abundant in vascular plants (Geissman and Crout, 1969) and were found abundantly in peat dominated by graminoids (Kuder et al., 1998; Schellekens et al., 2012). Furthermore, ester-bound vanillic acid is found in high amounts in seagrasses (Opsahl and Benner, 1993). The negative loading on F1 is then explained by the rapid degradation of non-lignin phenolic monomers (Section 3.2.4).

Both acetyl ratios, parameters 3c and 3d, were positively correlated (r^2 0.86, not shown) and showed evidently higher values during a wet period that was assigned to the Little Ice Age (Fig. 5; Schellekens et al., 2011). Both showed a similar fractionation of the communality with negative loading on both F1 and F4 (Figs. 2, 4). Their similar

behaviour indicates that they increased under both relatively wet (negative on F1; Fig. 2a) and secondary dry conditions (negative on F4; Fig. 2b), which may explain the absence of spikes in their depth profiles (Fig. 5). The clearly higher values during the Little Ice Age are explained by the duration of prevailing wet conditions. The initial increase in parameters 3c and 3d (upper 14 cm) suggest that the acetyl side chain is a result of anaerobic decay and cannot be interpreted as preservation due to the absence of aerobic decay. Thus, the formation of acetyl side chains occurs when the first step of aerobic decay is negligible and fresh material is subjected first to anaerobic conditions.

For the S moieties, the abundance of C₃ syringols (parameter 4b) was negatively correlated with parameter 3d (r^2 0.74, not shown), which suggests that C₃ syringols were transformed to 4-acetylsyringols during anoxic conditions at the first stage of decay. Anaerobic conditions during the first stage of decay (F1) and secondary aerobic decay (F4) explain the major part of the variance of parameter 4b (Fig. 4). Depletion of lignin moieties with a C₃ alkyl side chain also correlated with an increase in 4-acetylsyringol and 4-acetylguaiacol in peat investigated by Kuder and Kruege (1998). Although the effect of F4 dominated (Fig. 4), the abundance of C₃ syringols was obviously lower in the peat sections corresponding to the Little Ice Age (Fig. 5). A low abundance of C₃ syringols under wet conditions is not in agreement with the literature (Kuroda, 2000; van der Hage et al., 1993), as high abundances of lignin moieties with C₃ alkyl side chains are commonly interpreted as being from relatively intact lignin and thus indicating wet conditions (Philben et al., 2013).

Unlike the S moieties, for G moieties no correlation was found between C₃ and acetyl- alkyl side chains (r^2 0.01 for parameters 4a and 3c). C₃ guaiacols were mainly explained by F4 (Fig. 4) and showed particularly high values in the upper two samples (Fig. 5), indicating rapid decay of C₃ guaiacols. These rapidly degraded C₃ guaiacols

probably originate from graminoids (Schellekens et al., 2012). Nevertheless, a clear maximum for parameter 3c occurred during the Little Ice Age. This negative correlation between lignin moieties with C₃ alkyl and acetyl side chains, suggests that the former are oxidised during anaerobic conditions. This interpretation is supported by the good correlations of other lignin pyrolysis products with an oxidised side chain with parameters 3c and 3d. These include a correlation of syringic acid methyl ester (Lg38) with parameter 3d (r^2 0.84); vanillic acid methyl ester (Lg21) with parameter 3c (r^2 0.57); and 4-(propan-1-one)guaiacol (Lg23) with parameter 3c (r^2 0.55; not shown). It is not clear which mechanisms underlie these results.

A possible explanation is that, during prevailing wet conditions, the undecomposed material, still rich in easily degradable polysaccharides, is anaerobically degraded by bacteria. During the first step in anaerobic cellulose degradation various simple O-containing compounds are released (Béguin and Aubert, 1994), which might be used for the oxidation of lignin alkyl side chains in non-enzymatic Fenton based reactions as has been found for demethylation of wood lignin by brown rot fungi (Sinsabaugh et al., 2010; Filley et al., 2002).

3.2.4. Non-lignin phenolic acids, hemicellulose and cellulose (parameters 5-7)

Bourdon et al. (2000) used the ratio of cinnamic acids (i.e. ferulic acid and *p*-coumaric acid) to lignin as an indicator of Cyperaceae. Typical pyrolysis products of *p*-coumaric and ferulic acid are 4-vinylphenol and 4-vinylguaiacol, respectively (Boon et al., 1982; van der Hage et al., 1993). From analysis of peatland plants it appeared that 4-vinylguaiacol, from ferulic acid, was particularly associated with graminoids (parameter 5b), while 4-vinylphenol from *p*-coumaric acid (parameter 5a) showed a higher abundance in graminoids that perform better under wet conditions (Section 2.2). Also

ericoids showed a relatively high abundance of 4-vinylphenol (Schellekens et al., 2012). However, 4-vinylguaiacol and 4-vinylphenol also originate from intact lignin (Harman et al., 2013) and chain length reduction of C₃ alkyl side chains during decay, which occurred under both aerobic and anaerobic conditions (Schellekens et al., 2012). Therefore, the presence of phenolic acids cannot be evaluated properly via pyrolysis-GC-MS without methylation (del Rio et al., 2007) and the interpretation of 4-vinylguaiacol and 4-vinylphenol can be problematic.

Xylose, which is abundant in hemicellulose of grasses (Wende and Fry, 1996; Smith and Harris, 1999) and sedges (Bourdon et al., 2000), results in 4-hydroxy-5,6-dihydro-(2*H*)-pyran-2-one upon pyrolysis (parameter 6; Pouwels et al., 1987). The main pyrolysis product from cellulose is levoglucosan (Pouwels et al., 1989), of which non-lignified cellulose is reflected by parameter 1b and lignified cellulose by parameter 7.

The variance of parameter 5b is mostly explained by F3 (64%; Fig. 4) and is related to graminoids (Fig. 2b), while the explained variance of parameter 5a is more distributed among other factors. Ferulic acid and *p*-coumaric acid are rapidly degraded once the plant dies (Kuder and Kruege, 1998; Bourdon et al., 2000). A large part of both compounds is presumably already lost before it enters the peat. A smaller part is more resistant to decay (Tareq et al., 2006), which agrees with the maxima in parameters 5a and 5b during wet periods, except for a minimum during the Little Ice Age of parameter 5b (Fig. 5). These clearly lower values of 4-vinylguaiacol may indicate that ferulic acid was transformed to 4-acetylguaiacol under initial persistent wet conditions, as suggested in Section 3.2.3 for lignin C₃ alkyl side chains. Anaerobic degradation of ferulic acid can occur, with acetate being formed as an intermediate during metabolism (Healy et al., 1980), which supports this idea. Because the Penido Vello peat is dominated by graminoids and vegetation shifts upon lowering of the water table may be more

prominent within graminoids than between ericoids and graminoids, the positive loadings of parameters 5a and 5b on F3 may also be related to graminoids that perform better under relatively wet conditions (*Eriophorum* and *Molinia*), as these showed particularly high values of non-lignin phenolic acids (Schellekens et al., 2012).

The most abundant polysaccharide pyrolysis product in the peat was levoglucosan (Appendix). The loss of polysaccharides during primary decay originates mainly from non-lignified cellulose, which is easier to access. Assuming that non-lignified cellulose is degraded as rapidly as other plant polysaccharides, levoglucosan expressed as percentage of the total polysaccharide pyrolysis products reflects lignified cellulose (parameter 7). The increase with depth in parameter 7 (Fig. 5) showed the relative accumulation of lignified cellulose in an anaerobic environment (van der Heijden and Boon, 1994; Huang et al., 1997; Disnar et al., 2008; Fig. 4). The separation between parameters 6 and 7 on F2 (Fig. 2a), indicates that, during long term anaerobic decay, hemicellulose is preferentially degraded over lignified cellulose. The slightly positive loadings of parameter 7 on F1 also indicates that levoglucosan is less depleted than other polysaccharide pyrolysis products during primary decay (Fig. 2a), indicating that a large part of the cellulose was lignified. The negative loading of parameter 7 on F4 shows that, during secondary aerobic decay, lignified cellulose is degraded.

3.3. Palaeoclimatic implications

The application of the parameters to reconstruct environmental conditions from peat records or to predict the effects of climate change related to the carbon stored in peatlands is intricate. The variation with depth of most of the parameters depended on several factors, the contribution of the different decay processes and vegetation changes

to each of them is indicated in Fig. 4. Their usefulness as proxies for indicating bog hydrology is related to aerobic decay at the surface.

3.3.1. Lignin and polysaccharide content (parameter 1)

Both polysaccharide and lignin content indicate bog hydrology at the moment of peat formation, and reflect the preferential decay of polysaccharides over lignin. The lignin/polysaccharide ratio may thus be a reliable indicator of bog hydrology (Tsutsuki et al., 1994; Schellekens et al., 2011; Broder et al., 2012).

3.3.2. Syringyl, guaiacyl and p-hydroxyphenyl moieties (parameter 2)

In the Penido Vello core, the S/G ratio was completely determined by decomposition (Section 3.2.2.1). However, interpretation is complex and highly dependent on prevailing decay phases. The decrease in G lignin during the first stage of decay and long term anaerobic decay is presumably related to preferential decay of non-lignin phenolic acids (Schellekens et al., 2012); an additional explanation is differential decay of plant parts that contain higher G content (leaves). During secondary aerobic decay the more resistant plant parts and macromolecular lignin are accessible and S is preferentially lost.

Although parameter 2c had the lowest explained variance, the major part was related to source, namely ericoids. This confirms that a high abundance of H moieties in ericoids as obtained from peatland plant analysis (Schellekens et al., 2012) can still be related to its source in peat, notwithstanding the graminoid dominance in the peat core. The contrast with the general assumption that a high abundance of H moieties in soils is related to graminoids (Thevenot et al., 2010), indicates that parameter 2c is not always a trustworthy indication of source material.

3.3.3. Alkyl side chain oxidation (parameter 3)

Side chain oxidation is generally related to fungal degradation and therefore used to indicate aerobic conditions in peat records. In the Penido Vello core, half of the variation in vanillic and syringic acid was related to ericoids, and the other half to non-lignin phenolic acids from graminoids preserved under anoxic conditions during the first stage of decay (Fig. 4). Thus, a high abundance of vanillic and syringic acid may indicate (i) relatively wet conditions during the first stage of decay or (ii) an ericoid source, and thus indirectly relatively dry conditions. The absence of a relationship with secondary aerobic decay may be due to the fact that, under anoxic conditions, lignocellulose from graminoids is degraded more rapidly than that from wood, suggesting that wood is not yet strongly degraded.

Similarly acetyl side chains were related to both relatively dry (secondary aerobic decay) and wet (first stage of decay) conditions. The fact that the effect of anaerobic decay was more prominent in the depth records (Fig. 5) may be due to the long duration of the Little Ice Age.

3.3.4. Alkyl side chain reduction (parameter 4)

A large part of the C₃ alkyl side chains is degraded during secondary aerobic decay. However, the interpretation of lignin moieties with a C₃ alkyl side chain to reflect intact lignin may lead to erroneous hydrological interpretation, as C₃ syringols in particular were depleted during wet periods. Neither of both parameters (4a and 4b) was correlated with bog hydrology at the time of peat formation.

3.3.5. Non-lignin phenolic acids (parameter 5)

The cinnamyl/G ratio, generally used to differentiate between non-woody and woody tissue (Hedges and Mann, 1979), may be adequate for anaerobic systems with large changes in vegetation type (e.g. Tareq et al., 2006). However, no such correlation was found for the Penido Vello peat (not shown), probably because most G lignin pyrolysis products were as similarly rapidly degraded as 4-vinylguaiacol (from ferulic acid). However, the ratio 4-vinylguaiacol to lignin and the ratio of 4-vinylphenol (from *p*-coumaric acid) to H moieties are good indicators for graminoids, particularly those that perform better under relatively wet conditions (*Eriophorum* and *Molinia*, Section 3.2.4), which is in agreement with their abundance in peatland plants (Schellekens et al., 2012). The association of parameter 5a with graminoids is possibly caused by the fact that H moieties were related to ericoids (Section 3.2.2.2).

3.3.6. Hemicellulose and lignified cellulose (parameters 6 and 7)

During the first stage of decay hemicellulose and cellulose are degraded at equal rates, but relatively slowly compared with other polysaccharides. During long term anaerobic decay, hemicellulose is preferentially lost over cellulose; this was also found for *Calluna* tissue in peat and is presumably related to protection of cellulose by lignin (van der Heijden and Boon, 1994), in agreement with the high abundance of cellulose derived pyrolysis products found in residue peat samples by Schellekens et al. (2012).

3.4. C/N ratio

Carbon content (corrected for ash, r^2 0.75) and C/N (r^2 0.52) were positively correlated with F1, while nitrogen content was negatively correlated (corrected for ash, r^2 0.50; Fig. 3). These data indicate that higher C/N values were found in more decomposed samples (Pontevedra-Pombal et al., 2004). This contradicts the frequent

use of C/N to reflect decomposition in *Sphagnum*-dominated peat (Malmer and Holm, 1984; Kuhry and Vitt, 1996). Although high C/N, after detrending from the long-term depth trend, corresponded to wet periods indicated by non-pollen palynomorphs in a nearby peatland with similar vegetation type (Martínez-Cortizas et al., 2007), the huge difference in variability of the C/N record in *Sphagnum* peatlands (high variability; e.g. Schellekens and Buurman, 2011) compared to graminoid-dominated peatlands (low variability), suggests that the variation of C/N is strongly influenced by vegetation type as pyrolysis parameters showed high variability in both vegetation types. The positive correlation of carbon with F1 presumably reflects the loss of polysaccharides during initial decay (Schellekens, 2013). High values of nitrogen in the upper part of the core were mainly responsible for the negative correlation of nitrogen and F1 (Fig. 3), probably reflecting the rapid recycling of nitrogen by plants in the aerobic layer.

4. Conclusions

Factor analysis applied to pyrolysates of bulk peat sampled at high resolution allowed to distinguish effects of environmental factors on the Penido Vello peat chemistry. Identified environmental factors included surface decay (both anaerobic and aerobic), depth related decay (anaerobic), vegetation shifts (graminoids vs. ericoids) and sub-surface decay (aerobic), with decreasing impact on peat chemistry.

Decay at the surface showed greatest influence on peat chemistry, and is best reflected in the lignin/polysaccharide and S/G ratios, indicating that the major change in peat chemistry at the surface is a loss of easily degradable polysaccharides and non-lignin phenolic guaiacyl moieties under aerobic conditions. Depth related anaerobic decay showed minor effects on most lignin parameters, and was only significant for polysaccharide related parameters, indicating a decrease of hemicellulose and an

increase of cellulose with depth, the latter reflecting lignified cellulose. Although secondary aerobic decay in the sub-surface was less prominent in the overall peat chemistry, for the lignin parameters it showed a higher impact than depth related anaerobic decay. Sub-surface decay resulted in a loss of S and G moieties with a C₃ alkyl side chain, while moieties with acetyl side chains increased. The allocation of the effects of different processes on lignin parameters in combination with depth records of high-resolution sampled peat allowed to determine the net effect on bulk peat, and their usefulness as decomposition proxies.

The lignin composition of the studied peat core was predominantly determined by decay. However, the dominance of graminoids was the main factor that determined the consequences of decay on the composition of lignin phenols and considerably differed from that of woody tissue. Our results support the idea that lignin decay is a surface process, and that anatomical structure or accessibility control the decay of lignocellulose at different scales (van der Heijden and Boon, 1994; Grabber et al., 1997, 2004; Love et al., 1998; Talbot et al., 2012). Because plant groups differ in chemistry a general decay mechanism may not be valid for peat composed of unknown mixtures of different plant groups (ericoids-graminoids-mosses). For the reconstruction of peat water surface wetness from lignin chemistry we recommend the use of a number of vegetation and decomposition proxies that provide similar information.

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